COMPARATIVE STUDY ON ALVEOLAR BONE HEALING IN POST EXTRACTION SOCKET VERSUS HEALING AIDED WITH AUTOLOGOUS PLATELET RICH FIBRIN FOLLOWING SURGICAL REMOVAL OF BILATERAL MANDIBULAR IMPACTED THIRD MOLAR TOOTH – A RADIOGRAPHIC EVALUATION

Dissertation submitted to

THE TAMILNADU Dr. MGR MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH III

DEPARTMENT OF ORAL AND MAXILLOFACIAL SURGERY

APRIL 2018

CERTIFICATE

This is to certify that this dissertation titled "**COMPARATIVE STUDY ON ALVEOLAR BONE HEALING IN POST EXTRACTION SOCKET VERSUS HEALING AIDED WITH AUTOLOGOUS PLATELET RICH FIBRIN FOLLOWING SURGICAL REMOVAL OF BILATERAL MANDIBULAR IMPACTED THIRD MOLAR TOOTH – A RADIOGRAPHIC EVALUATION"** is a bonafide record of work done by **Dr. N. SANTHOSHI REVATHY** under my guidance during her postgraduate study period of 2015-2018.

This dissertation is submitted to **THE TAMILNADU Dr. MGR MEDICAL UNIVERSITY,** in partial fulfilment for the degree of **MASTER OF DENTAL SURGERY** in Branch III- **ORAL AND MAXILLOFACIAL SURGERY.**

It has not been submitted (partially or fully) for the award of any other degree or diploma.

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I hereby declare that this dissertation entitled **COMPARATIVE STUDY ON ALVEOLAR BONE HEALING IN POST EXTRACTION SOCKET VERSUS HEALING AIDED WITH AUTOLOGOUS PLATELET RICH FIBRIN FOLLOWING SURGICAL REMOVAL OF BILATERAL MANDIBULAR IMPACTED THIRD MOLAR TOOTH – A RADIOGRAPHIC EVALUATION**" is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. KANNAN M.D.S., PROFESSOR DEPARTMENT OF ORAL AND MAXILLOFACIAL SURGERY, SRI RAMAKRISHNA DENTAL COLLEGE AND HOSPITAL, COIMBATORE.**

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Above all, I bow my head to Almighty!

Dr. N. Santhoshi Revathy.

LIST OF ABBREVIATIONS

PRP	Platelet Rich Plasma
PRF	Platelet Rich Fibrin
L-PRF	Leucocyte- Platelet Rich Fibrin
I-PRF	Injectable- Platelet Rich Fibrin
T-PRF	Titanium- Platelet Rich Fibrin
A-PRF	Advanced- Platelet Rich Fibrin
GF	Growth Factor
PDGF	Platelet Derived Growth Factor
VEGF	Vascular Endothelial Growth Factor
IGF	Insulin like Growth Factor
TGF-β	Transforming Growth Factor
EGF	Epithelial Growth Factor
cPRF	concentrated Platelet Rich Fibrin
IL	Interleukin
TNF-α	Tumor Necrosis Factor-α
BMSC	Bone marrow Mesenchymal Stem Cells
BMP	Bone Morphogenic Protein
RBH	Residual Bone Height
BAOSFE	Bone-added Osteotome Sinus Floor Elevation
DFDBA	Demineralized Freeze Dried Bone Allograft
FDA	Fluorescein Diacetate

SEM	Scanning Electron Microscopy
RBC	Red Blood Corpuscles
PI	Propidium Iodide
LDH	Lactate Dehydrogenase
TBT	Topical Bovine Thrombin
OPG	Osteoprotegelin
p-ERK	Phosphorylated Extracellular Signal Regulated Protein Kinase
ALP	Alkaline Phosphatase
GBR	Guided Bone Regeneration
HE	Hematoxylin Eosin
CAF	Coronally Advanced Flap
CTG	Connective Tissue Graft
LO	Localized Osteitis
RVG	Radiovisiography
OPG	Orthopantomogram
OFD	Open Flap Debridement
PD	Probing Depth
DBB	Deprotinized Bovine Bone
OD	Optical Density
HA	Histomorphometric Analysis
BHA	Bovine Hydroxyapatite
ASA	American Society of Anaesthesiologist
RBG	Red Blue Green

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INTRODUCTION

Wound can occur as part of a disease process, an accidental or intentional aetiology. Disruption of the integrity of skin, mucosal surfaces or organ tissue results in the formation of a wound. Wound healing is a complex biological process which results in the restoration of tissue integrity. At the time of insult, multiple cellular and extracellular pathways are activated, in a tightly regulated and coordinated fashion, with the aim of restoring tissue integrity [(1)].

The process of wound healing is divided into four distinct phases: hemostasis, inflammation, proliferation and tissue remodelling. Immediately after injury the hemostasis occurs resulting in vascular constriction and fibrin clot formation. At the end of first phase, inflammatory phase starts in which chemotaxis take place resulting in prevention of infection. In the third proliferative phase angiogenesis, granulation tissue formation, collagen deposition, epithelialization and wound retraction occurs and begins to repair. In the final phase of remodelling, development of normal epithelium and tissue maturation take place [(1)].

Platelets aggregated in the injured site influences wound healing right from the first phase. Platelets release growth factors locally, which are required for wound healing and bone regeneration. Attempts were made to increase the concentration of growth factors by the use of various platelet preparations introduced into the wound healing [(1) (2)].

In 1974, Ross et al., were the first to describe growth factor from the platelets trapped within a fibrin matrix, responsible for mitogenic response in the bone periosteum during normal wound healing [(3)]. In 1990 Gibble and Ness introduced fibrin glue, alternatively referred as fibrin sealant or fibrin gel. This biomaterial was developed in order to improve the hemostatic agents with adhesive properties [(4)].

Consequently, the use of fibrin glue was replaced by platelet concentrates to improve healing as first described by Whitman et al [(5)].Various uses and actions of Platelet concentrates

has been explored considerably during the last decade. Platelets contain high quantities of key growth factors, such as PDGF-AB (Platelet-Derived Growth Factor AB), TGFβ-1 (Transforming Growth Factor β-1) and VEGF (Vascular Endothelial Growth Factor), which has the ability to stimulate cell proliferation, matrix remodelling and angiogenesis [(6).]

Platelet-rich plasma (PRP) is an autologous concentrate and it contains relatively small volume of plasma, which enables delivery of growth factors in increased amounts to surgical sites. They promote the wound healing and stimulate collagen production, improve wound strength, and initiate callus formation. PRP gel is formed by mixing PRP derived from centrifugation of autologous blood, with topical bovine thrombin (TBT) and calcium chloride. PRP gel has a high concentration of platelets and fibrinogen. Calcium chloride triggers the platelet activation and fibrin polymerization [(7).]

The introduction of an endogenous initiator of coagulation (usually bovine thrombin), in most available methods of PRP preparation has the effect of causing rapid degranulation of platelets and almost immediate liberation of growth factors into the surgical area at the time of preparation. Since growth factors released by PRP have a limited time of effectiveness, it can only affect the immediate stages of wound healing and not for the extended period of time needed for bone and soft tissue regeneration [(8)].

The TBT has been reported to be linked with the development of antibodies to factors V, XI and thrombin resulting in the risk of life-threatening coagulopathies. TBT preparations contain factor V, which results in reaction of the immune system when challenged with a foreign protein. The factor V deficiency after thrombin exposure is caused by the cross-reactivity of anti-bovine factor V antibodies with human factor V [(4)].

To overcome the disadvantages of PRP, a new family of platelet concentrate was introduced in France by Choukroun called Platelet Rich Fibrin (PRF). Platelet Rich Fibrin is considered as second generation platelet concentrate, because the natural concentrate is produced without any anticoagulants or gelifying agents.

Platelet Rich Fibrin is a fibrin matrix in which platelet cytokines, growth factors and cells are trapped and released gradually over a period of time. PRF can serve as a resorbable membrane and is used in improving bone healing [(9)]. It consists of a fibrin matrix polymerized in a tetra molecular structure, with incorporation of platelets, leucocytes, cytokines and circulating stem cells in to the matrix. Autologous Platelet Rich Fibrin is considered to be a healing biomaterial which will accelerate the physiologic wound healing and new bone formation. Platelet activation and fibrin polymerization are triggered without addition of anticoagulant [(10)].

PRF has been most widely used in cardiac surgery and vascular surgery to seal diffuse microvascular bleeding. It is also used to seal wound borders which facilitate the cutaneous reuse in general and plastic surgery. In Oral and Maxillofacial Surgery PRF is used in sinus lift procedures, implant procedures, alveolar osteitis, extracted sockets and cyst enucleation procedures [(11)].

Platelet Rich Fibrin has following advantages [(3)]:

a) Simple and easy to prepare.
b) The preparation of PRF takes less time compared to PRP.
c) No biochemical handling of blood.
d) Simplified and cost effective process and use of bovine thrombin and anticoagulants avoided.
e) More efficient cell migration and proliferation.

f) Favorable healing due to polymerization.

g) PRF has supportive effects on immune system.

h) PRF helps in hemostasis.

In our study, we evaluated the efficacy of PRF in wound healing by comparing bone healing in sockets packed with PRF with that of sockets allowed to heal normally, following bilateral surgical removal of impacted third molar performed on the same day by the same surgeon. Patient was recalled for follow up after 1stmonth, 3rdmonth and 6thmonth postoperatively to assess the difference in bone formation by measuring the density on both the sides with the help of Orthopantomogram (OPG). For bone density measurement we used image processing tool box from Matlab software with OPG as an input. Using the image processing tool box density measurement was done both in the region of the PRF placed socket and control socket by extracting the pixels across the selected region. The pixel values were measured in grey scale and the tool box in turn analyzes the pixel intensity to infer the density value. Values from both the sides were compared and analyzed to find the side that has attained better wound healing.

AIMS & OBJECTIVES

AIM:

- To evaluate the potential of Autologous Platelet Rich Fibrin in improving the alveolar bone healing and bone regeneration after placement of PRF in one of the sockets following bilateral surgical removal of impacted mandibular third molar and comparing with the alveolar bone healing and bone regeneration in the opposite side socket closed primarily without PRF.

OBJECTIVES:

- To evaluate the efficacy of Platelet Rich Fibrin in the healing of surgical extraction wound.
- To evaluate the efficacy of Platelet Rich Fibrin in bone regeneration and maturation in the extraction site.
- To evaluate the role of PRF in minimizing the post-operative complications following surgical extraction of a tooth.
- To provide safe, faster, and economical method of obtaining Autologous Platelet concentrate without artificial additives.

REVIEW OF LITERATURE

DAVID R. KNIGHTON, THOMAS K. HUNT et al (1982) [12] investigated the capacity of platelets and fibrin to initiate angiogenesis, fibroplasia and collagen synthesis and monocyte migration in the rabbit cornea assay. In their study, autologous platelets and platelet-free fibrin were isolated from rabbit blood. Autologous and commercial fibrins were implanted in rabbit corneas. The fibrin released from platelet act as an initiator for cellular response to promote wound healing and it is also responsible for early burst of fibroplasia, collagen synthesis and neovascularization. Fibrin and its degradation product, along with macrophages and monocytes circulate in the wound site and produce necessary growth factors and chemoattractant until repair is complete. They conclude that Thrombin- activated platelets produced angiogenesis, fibroplasia and increase collagen synthesis. Histological examination showed fibroplasia, corneal thickening, and neovascularization. Collagen synthesis was elevated twice to control the levels in thrombin-activated platelet preparations.

ANDRES R. SANCHEZ et al (2003) [4] revealed that PRP gel contains high concentration of platelets and fibrinogen. The platelet concentrate is activated by the addition of thrombin and calcium chloride and this results in the release of a cascade of growth factors from the platelet alpha (α) granules and this growth factors have important roles in the regulation of growth and released various growth factors such as platelet-derived growth factor, transforming growth factor β, platelet-derived epidermal growth factor, platelet-derived angiogenesis factor, insulin-like growth factor 1, and platelet factor 4. These factors signal the local mesenchymal and epithelial cells to migrate, divide and increase collagen and matrix synthesis. When PRP is mixed with bone autografts and allografts it resulted in rapid epithelialization and formed dense and mature bone with better organized trabeculae and greater bone regeneration. PRP also accelerates soft tissue healing by promoting a more rapid revascularization and

reepithelialization of flaps and cell proliferation. PRP delivers a highly concentrated dose of autologous platelets containing a variety of biologic mediators. PRP can be applied directly to the healing site which increases the rate of bone deposition and quality of bone regeneration when augmenting sites prior to or in conjunction with dental implant placement.

J. J. THORN, H. SORENSEN, U. WEIS-FOGH et al (2004) [13] described a method for the preparation of autologous fibrin glue with platelet growth factors and reported their use with particulate cancellous bone in reconstructive maxillofacial surgery. The fibrin glue consists of two-component glue, where one component is a concentrated fibrinogen solution with platelet growth factors and the other component is a thrombin solution. Both components were produced from the patient's own blood, thus making the glue entirely autologous. They combined the high concentration of fibrinogen and growth factors and the fibrinogen concentration was 12 times the value as found in PRP. Platelet Derived Growth Factor was 8 times the value found in PRP. The healing process is enhanced by incorporating blood platelets into the fibrin glue. The growth factor such as PDGF and TGF-β are released from platelets upon activation with thrombin. In combination with IGF which is located in plasma and bone morphogenic proteins located in bone tissue have closest association to bone regeneration. The prepared autologous fibrin glue was mixed with bone graft and used to reconstruct the lost parts of mandible, with osteoradionecrosis and ameloblastoma. They concluded that the glue accelerates the bone graft healing process, as the fibrin network is known to act as a scaffold for the invasion of cells and as a carrier for bone induction. The glue contains high concentration of fibrinogen, growth factors and thrombin which produce sufficient strength to cause rapid coagulation.

DAVID M. DOHAN et al (2006) [14] Part-I series conducted a study on technological concepts and evolution from fibrin glue to platelet concentrate. The development of new platelet

concentrate offers simplified and optimized production of new kind of fibrin adhesives called cPRF. Because of legal restrictions on blood handling new family of platelet concentrate appeared in France called PRF, it looks like an autologous cicatricial matrix. They evaluated the biochemical properties of three generations of surgical additives, the fibrin adhesives, concentrated platelet-rich plasma (cPRP) and PRF. They reported the main differences between fibrin adhesives, cPRP and PRF attributable from the gelly mode. Fibrin adhesives and cPRP use calcium chloride and bovine thrombin to commence the last stage of coagulation and sudden fibrin polymerization. This mode of polymerization will influence the mechanical and biological properties of fibrin matrix. PRF has the characteristic of natural polymerization occurring slowly during centrifugation and the thrombin concentrations acting on the collected autologous fibrinogen are almost physiologic because there is no bovine thrombin. Thus the slow polymerization during PRF preparation will form fibrin network similar to natural one leading to more efficient cell migration, proliferation and cicatrization. In contrast, weak thrombin concentration implies significant percentage of equilateral junctions. These connected junctions allow the establishment of fine and flexible fibrin network able to support cytokine enmeshment and cellular migration. In this three dimensional organization, PRF gives great elasticity, flexibility and a stronger membrane.

DAVID M. DOHAN et al (2006) [15] **Part-II series** studied on Platelet-related biological features of the platelet rich fibrin (PRF) material. During PRF processing by centrifugation, more platelet accumulates mainly between the RBC and fibrin clot from which the platelets are activated and their massive degranulation releases cytokines. These cytokines are found neither in the supernatant nor in the exudates. They remain trapped in the PRF fibrin matrix even after serum exudation which implies incorporation of these molecules in the fibrin polymer molecular

architecture. Some growth factors released from the platelets such as TGFβ-1 have the capacity to induce fibrous cicatrization through inflammatory regulators and PDGF play a role in mechanism of physiologic cicatrization and pathogenesis of atherosclerosis and other fibroproliferative diseases. A progressive polymerization mode significantly increases the incorporation of the circulating cytokines in the fibrin meshes which implies an increased lifespan for these cytokines, because they will be released and used only at the time of initial cicatricial matrix remodelling. During slow polymerization the physiologic thrombin concentrations implies elastic matricial architecture. The intimate assembly of cytokines, glycanic chains, and structural glycoproteins are enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergetic effects on healing processes. They consider the PRF as a healing biomaterial more than a new kind of fibrin biological adhesive.

DAVID M. DOHAN et al (2006) [16] **IN THEIR SERIES OF PART III:** investigated the immune features of this biomaterial by examining the leucocyte activation. During PRF processing leucocyte degranulation take place with release of cytokines ranging from proinflammatory mediators, such as IL-lb, IL-6 and TNF-α, to anti-inflammatory cytokines, such as IL-4 and VEGF. They concluded that PRF is not only a platelet concentrate but also an immune node able to stimulate defense mechanisms with significant inflammatory regulation noted on surgical site treated with PRF in the outcome of retrocontrol effects from cytokines trapped in the fibrin network and released during remodeling of this initial matrix.

DAVID M. DOHAN et al (2006) [17] **IN THEIR SERIES OF PART IV:** The fibrin is used as a matrix for transplantation of mesenchymal stem cells because the mesenchymal stem cells from bone marrow contribute to the regeneration of whole type bone cells and other tissues. These

undifferentiated cells are recruited from blood to injured tissues and it differentiated into several different cell types. Fibrin and fibronectin is formed by initial differentiation occurring in a transitory scar matrix. The fibrin is recognized as support matrix for bone morphogenetic protein transplants. The fibrin matrix associated with BMP has angiotrophic, hemostatic and osseous conductive properties.

CHOUKROUN et al (2006) [18] **IN THEIR SERIES OF PART V:** evaluated the potential of Platelet Rich Fibrin with freeze-dried bone allograft (FDBA) to enhance bone regeneration in sinus floor elevation by histologic analysis. They carried a study on nine sinus floor augmentations. In three sites FDBA without PRF was used (control group) and in six sites, PRF was added to FDBA particles (test group). The PRF clots were recovered and tightly packed in between 2 sterile gauzes and compressed in order to obtain resistant fibrin membrane transferable to the schneiderian membrane, in order to prevent or treat perforation and placed as a membrane on the graft material before wound closure. PRF is used with FDBA in sinus floor augmentation to accelerate bone regeneration and allow implant placement after 4 months of healing. The healing time between sinus graft and implant placement could be reduced using PRF. PRF combined with bone graft material is attractive from histologic point of view.

SHI-JIANG ZHU et al (2006) [19] conducted a study to compare the combined effects of platelet-rich plasma (PRP) and BMSC with platelet-enriched fibrin glue and BMSC for bone formation in bone tissue engineering in mice. Platelet enriched fibrin glue is used as a scaffold because it contains high concentration of fibrinogen, which can produce dense fibrin clot with sufficient strength to maintain a required configuration. PRP was mixed with bone marrow mesenchymal stem cells and bone morphogenetic protein-2 (BMP-2) and the composites were injected on the dorsum of nude mice into the subcutaneous space. Fibrin glue offers a benefit in

accelerating wound healing by the presence of high concentration of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF-β) and vascular endothelial growth factor (VEGF). These growth factors promote cell proliferation, cell differentiation, motility and matrix synthesis either alone or binding with specific cell surface receptor. On the control side, the platelet-enriched fibrin glue/bone marrow mesenchymal stem cells/BMP-2 composites were injected on the dorsum. After twelve weeks histological examination of nodules revealed that they were encapsulated with a fibrous capsule and there was a trabecular bone seen in more volume in PRF side and less volume in control side. Bone formation was evaluated using image analysis system after 12 weeks. They concluded that the osteogenic characteristics of platelet-enriched fibrin glue are superior to PRP in bone tissue engineering.

ANTOINE DISS, DAVID M. DOHAN, JAAFAR MOUHYI et al (2008) [(20)] documented the changes in the apical bone levels radiographically on micro-threaded implants placed in sub-sinus residual bone, following bone-added osteotome sinus floor elevation technique grafted with platelet-rich fibrin (PRF). Residual bone height (RBH) under the maxillary sinus during implant placement and the change in endosinus bone level at one year was determined by radiographic analysis. The survival rate at abutment tightening was done in 6 to 12 weeks of healing. They found that the BAOSFE procedure with PRF as grafting material can create a space for predictable bone formation beyond the sinus floor and lead to an endosinus bone gain of 3.2 mm on average. The healing period of 2-3 months was found to be sufficient to resist a torque of 25 cm applied during abutment tightening and the survival rate of these implants was 97.1% in one year. At one year, formation of new bone structure delimiting the sinus floor seen radiologically lead to predictable implant function. In this OSEF procedure, PRF used as a grafting material

have the following advantages 1. There is no need for donor site. 2. Limited perforation can be treated because of healing capacity of fibrin matrix. 3. There is no risk of sinus infection, if the grafting material is penetrated into the sinus. 4. There is no need to compromise because it is more affordable. 5. The material protects the membrane from perforation by direct contact with metallic osteotome. 6. The use of filling material permits a larger distension of the membrane and preparation of a larger grafted volume.

B.I. SIMON, A.L. ZATCOFF, J.J.W. KONG et al (2009) [(21)] performed a canine study to determine clinical and histological comparison of extraction socket healing with the use of autologous Platelet-Rich Fibrin Matrix (PRFM) with and without demineralised freeze dried bone allograft material covered by resorbable collagen membrane. Soft tissue healing appeared to be more rapid in the PRFM alone sites. Histologically, the healing was quite different and rapid for those sockets treated with PRFM alone or with a membrane as compared to those sites in which DFDBA was used as a graft. By three weeks sockets were osseous filled. Sites containing DFDBA had little new bone by 6 weeks. By 12 weeks only those sockets had osseous fill but DFDBA particles were still noted in coronal areas.

PUSHKAR D. GAWANDE, RAJSHEKAR HALLI et al (2009) [(22)] conducted a study on efficacy of platelet rich plasma on bone regeneration. Platelets have many functions beyond that of simple hemostasis. It contain important growth factors responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating growth and inducing cell differentiation. These are crucial steps in early wound healing. Using this concept the increasing concentration of platelets at the wound site may promote more rapid and better healing. The increasing concentration of platelets in the bone graft and the growth factor may lead to more rapid and denser bone regeneration.

ZIV MAZOR, ROBERT A. HOROWITZ, MARCO DEL CORSO et al (2009) [23] assessed a study about the relevance of PRF clots and membranes as the sole filling material during 25 lateral sinus lift with immediate implantation, using radiologic and histologic analyses using biopsy 6 months after the procedure. They evaluated the subsinus residual bone height and final bone formation around the implants. In 9 patients, after 6 months of sinus lift procedure bone biopsies were done on the buccal wall of alveolar ridge and histomorphometry were evaluated. They concluded from a radiologic and histologic point of view at 6 months after surgery, that the observed bone must be considered new bone built starting from the sole PRF fibrin matrix. At a low magnification, the architecture of the bone looked natural, with structured trabeculae and a dense collagen matrix. At a high magnification, osteoblasts were easily identified and osteocytes in the lacunae demonstrated the vitality of this bone sample. The PRF used as the sole filling material during a simultaneous sinus lift procedure and implantation were stabilized with a high volume of naturally regenerated bone in the subsinus cavity up to the tip of the implants. All biopsies showed well organized and vital bone formation.

ANTHONY P. SCLAFANI (2009) [24] revealed the use of PRFM in facial applications. Autologous platelet derivative allows rapid and inexpensive generation of a selphyl platelet rich fibrin matrix (PRFM) that can be used to enhance healing after facial procedures as well as to rejuvenate the face without tissue manipulation. Selphyl (Aesthetic Factors, LLC, Princeton, NJ) is an FDA-cleared device consisting of materials needed to produce an autologous PRFM. This PRFM is capable of sustained release of PDGF-BB, VEGF-A, TGF-b, and IGF-1 over 7 days with increased endothelial cell proliferation. It provides autologous, natural but concentrated platelet growth factor release and stimulates the surrounding tissue. After the platelet-fibrin suspension is mixed with calcium chloride, the activated PRFM can be sprayed under a skin or

myocutaneous flap to promote hemostasis, fibrosis and angiogenesis. Likewise, after positioning of facial implants PRFM is placed in the pocket to accelerate soft tissue enclosure and in growth into porous implants. PRFM is also used in the cases of dermal augmentation, acne scar removal and autologous fat transfer.

CHEN YAO SU, YA PO KUO et al (2009) [25] determined the use of growth factors released from PRF and their clinical applications. Growth factors promote hard and soft tissue repair mechanism and exhibit chemotactic and mitogenic properties that promote cellular function in tissue healing, regeneration and cell proliferation. Growth factor released by α-granules encompasses a group of cytokine polypeptides PDGF, TGF-β, VEGF, EGF and IGF-1. It exerts chemotactic effects towards osteoblast. They concluded that GF were released from PRF within 300 minutes and also supernatant serum has a stable GF content over 300 minute study period, which indicates that it is fully depleted of platelets. PRF clots should be squeezed between sheets of cotton gauze to obtain a fibrin membrane, the resulting fluid was discarded. The fluid that remains after membrane formation was also used to mix with bone graft. The PRF should be used within the 1st hour. There would be continuous release of the GF during initial healing period after application of the membrane on the surgical site.

DAVID M. DOHAN EHRENFEST, MARCO DEL CORSO et al (2010) [26] conducted this study to determine the cell composition and three-dimensional architecture of PRF and to evaluate the influence of different collection tubes (dry glass or glass-coated plastic tubes) and compression procedures (forcible or soft) on the final PRF-membrane architecture. Blood analyses were performed after centrifugation, on the residual waste plasmatic layers after collecting PRF clots. The PRF clots and membranes were processed by light microscopy and scanning electron microscopy for examination. The photonic microscopy study showed that the

platelet and leukocyte distribution within the clot was not uniform. The platelet and leucocytes concentration were located intermediately between RBC and fibrin clot that represent buffy coat on PRF surface. So we have to preserve the small RBC layer while taking PRF clot. Platelet counts clearly showed any platelet left within the RBC layer, the PPP or the exudate provided by compressing the PRF clot. Thus most of the platelets originating from the whole-blood sample were collected in the PRF membranes. In Scanning Electron Microscopic examination, leukocyte counts confirmed that more than half of the leukocytes were trapped in PRF membranes and small lymphocytes were collected. They showed the results as approximately 97% of the platelets and >50% of the leukocytes were concentrated in the PRF clot and they showed a specific three-dimensional distribution, depending on the centrifugation forces. The fibrin network was very mature and dense clusters due to extensive aggregation and it formed a large cluster of coagulation in the first few millimeters of the membrane beyond the red blood cell base. They concluded that there was no significant difference in the PRF architecture by using different collection tubes and by compression techniques.

EUN-SIK JANG, JUN- WOO PARK et al (2010) [27] determined the capability of silk fibroin powder mixed with PRF in the restoration of peri-implant defects. Silk protein is enzymatically degradable and biocompatible and was used as a scaffold along with PRF for the restoration of bony defects. After placing immediate implants, the silk fibroin with PRF was placed in the peri-implant bony defects. In the histomorphometric results they showed greater bone formation in the PRF side compared to control side.

BAHADIR GURBUZER, LEVENT PIKDOKEN et al (2010) [28] described the scintigraphic evaluation on the early healing process in the extraction sockets treated with PRF based on technetium-99m diphosphonate. Fourteen patients were included in the study with bilateral soft

tissue impacted third molars. All the patients in this study with bilaterally impacted third molars were surgically extracted in one session. PRF was randomly administered into one of the extracted sockets, whereas the contralateral sockets were left without PRF. Scintigrams were obtained 4 weeks after surgery to evaluate difference between PRF-treated and non-PRF-treated sockets. All the PRF-treated sockets healed uneventfully and postsurgical infection did not occur in any of these sites. Their results demonstrated that the early bone formative changes in PRF-treated sockets were almost equal to that in non- PRF-treated sockets 4 weeks after surgery. They concluded that four weeks after surgery PRF might not lead to enhance the bone healing in soft tissue impacted mandibular third molar extraction socket and the PRF exhibits potential characteristics of an autologous fibrin matrix.

VOLKCR GASSLING, TIMOTHY DOUGLAS et al (2010) [(29)] compared PRF with the collagen membrane. Bio-Gides used as scaffolds for periosteal tissue engineering. Human periosteal cells were seeded on membrane pieces in the collagen [Bio-Gide] and PRF at a density of 104cells/well. The cell vitality was assessed by fluorescein diacetate (FDA) and propidium iodide (PI) staining, biocompatibility with the lactate dehydrogenase (LDH) test and proliferation level with the MTT, WST and BrdU tests and scanning electron microscopy (SEM). Their results showed that assessment of cell vitality by staining indicate that no cells died as a result of elate from membranes. They showed higher level of biocompatibility of PRF membrane, as shown by the LDH test. The metabolic activity measured by the MTT and WST tests was higher for PRF, when compared to collagen (Bio-Gides). The proliferation level as measured by the BrdU test (quantitative) and SEM examinations (qualitative) revealed higher values for PRF than for collagen. They concluded that PRF appears to be superior to collagen (Bio-Gides) as a scaffold

for human periosteal cell proliferation and are suitable for in vitro cultivation of periosteal cells for bone tissue engineering.

RUCHI PATHAK, SUHAS et al (2011) [5] they evaluated the use of autologous platelet rich plasma (PRP) to promote wound healing and osseous regeneration in human third molar extraction sockets. Platelet-rich plasma (PRP), an autologous concentrate of platelets in a small volume of plasma, enables delivery of growth factors in increased amounts to surgical sites, to promote wound healing. Platelets contain growth factors such as platelet derived growth factor (PDGF), transforming growth factor-b (TGF-b), fibroblast growth factor, insulin-like growth factor-I (IGF-I), epithelial growth factor, vascular endothelial growth factor and numerous other secretory proteins. PDGF and TGF-b improves the soft tissue and bone healing, stimulate collagen production, improve wound strength and initiate callus formation. The PRP reduces the wound dehiscence, signifies a better soft tissue healing of extracted socket when compared to non PRP side. A significant difference was seen in the density measurement of alveolar bone level in the PRP placed extracted socket.

BARRY I SIMON, PRIYU GUPTA, SHEREEN TAJBAKSH (2011) [30] evaluated the extraction socket healing with the use of autologous platelet rich fibrin matrix in humans. Their study population consisted of 21 subjects who required tooth extraction and a GBR procedure, followed by implant placement. They evaluated the alveolar height and width, measured by inserting a patient- dedicated UNC Williams periodontal probe (Hu-Friedy) at three specific time intervals immediately following tooth extraction, after ridge augmentation and after 4 months of healing, at the time of implant placement. In their investigation they found that after 4 months of healing, the sockets were filled with mature bone 5mm apical to crest. The dimension of alveolar ridge was completely preserved during healing but there is minimal net loss of width and height.

Sites grafted with PRFM show rapid clinical healing, minimal flap re-opening and excellent bone density. The use of PRFM alone was found to be advantageous which include- less surgical time and potential healing difficulties associated with membranes and less resorption during healing, when compared to guided bone regeneration procedures.

Y-C CHANG, J-H ZHAO et al (2011) [(9)] described the effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects. PDLFs were derived from healthy individuals, who had undergone extraction for orthodontic reasons. The effects of PRF on PDLFs were determined by measuring the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK), osteoprotegerin (OPG) and alkaline phosphatase (ALP) activity. They found that PRF increased the ERK phosphorylation in human osteoblast and OPG protein expression in human osteoblast cell line and pulp cells in PDLF at day 1, 3, and 5, respectively and the ALP activity was also significantly upregulated by PRF. Application of PRF in infrabony defects exhibited pocket reduction and clinical attachment gained after six months. They concluded that the enhancement of p-ERK, OPG and ALP expression by PRF may provide benefits for periodontal regeneration and the use of PRF is an effective modality for periodontal infrabony defects. After 12 months follow up the application of PRF was exhibited by radiographic evidence that the defect was filled with bone-like dense tissue in the furcation areas.

M. DEL FABBRO, M. BORTOLIN et al (2011) [(31)] studied the use of autologous platelet concentrate to promote the healing of extraction socket. Several techniques enhance the regeneration process in order to reduce alveolar bone dimensional changes in the extraction socket such as autogenous bone grafts or bone substitutes, guided bone regeneration (GBR) with resorbable or non-resorbable barriers, enamel matrix derivative, recombinant growth and

differentiation factors and autologous platelet concentrates. The autologous platelet concentrates achieves the potential of several platelet growth factors (PDGF, TGF-beta, EGF, VEGF, IGF-I, b-FGF, HGF) to stimulate chemotaxis, angiogenesis, proliferation, differentiation, modulation for rapid and effective regeneration of hard and soft tissues. Platelets also play a host defense mechanism at the wound site, by delivering the signalling peptides which attract macrophage cells. Platelet concentrates may contain small amounts of leukocytes that synthesize interleukins involved in the non-specific immune reactions. The platelet concentrates reduced the post-operative pain and inflammation and accelerated the hard tissue regeneration but it did not accelerate the soft tissue healing in the post extraction socket. In the infected sockets hard tissue regeneration developed more slowly compared with disease-free sockets.

OLUFEMI K. OGUNDIPE, VINCENT. I et al (2011) [32] investigated the bone regeneration potential, post-operative pain, swelling, trismus, as well as healing on mandibular third molar socket by using Platelet Rich Plasma gel. They did a comparative clinical study on surgical extraction of single impacted third molar of about 60 patients in 2 years. They placed PRP gel in the socket of third molar in test group whereas the no PRP gel was placed in control group. The postoperative pain was measured using visual Analog scale. The facial swelling, interincisal mouth opening, trismus were lower in test group compared to control group. PRP has its ability to initiate and stabilize blood clot in extraction socket and it induced faster bone formation.

TZONG-FU KUO, ET AL (2011) [33] investigated the regeneration of articular cartilage defects of the knee of rabbits following the implantation of PRF with cartilage granules in a one-step procedure using T2-map magnetic resonance imaging (MRI) and histology to assess the rabbits' response to the procedure. A total of 12 New Zealand white rabbits were divided into two groups. In group A the cartilage defect was created with no implantation. In group B cartilage

defect was created, and implanted with a mixture of PRF and cartilage granules. After three months of surgery, the animals were scanned using MRI. The T2 value for each rabbit in group A and group B was compared with that of the same rabbit's normal cartilage on the contralateral side. After sacrificing the animal, all experimental limbs were dissected, the specimens were embedded in paraffin, sectioned and processed for routine hematoxylin-eosin (HE) staining, recommended by the ICRS to assess the degree of cartilage regeneration. In their study control animal's defective cartilage matrix become more fibrotic which reduce the T2 value. The regenerated cartilage of treated animals has higher T2 value than defective cartilage, indicating a decrease in proteoglycans and a progressive increase in collagen content. They hypothesize that PRF mixed with cartilage granules provides favourable conditions for cell migration and cell growth. The use of PRF and cartilage granule without bovine thrombin represents a one-step cartilage repair surgery with potentially favourable results.

ATHRAA Y. ALHIJAZI, SIBA A. MOHAMMED et al (2011) [(34)] evaluated the effect of platelet rich fibrin matrix as bone filler of socket after tooth extraction by histologically and radiographically. Twenty four rabbits were used in their study and they extracted upper central incisors under general anesthesia .The left side was filled with platelet rich fibrin matrix material and the right side was taken as control group. Histologically Autologous platelet rich fibrin matrix in the extracted socket shows the formation of neovascularization and more rapid and faster apposition of bone matrix with its mineralization process. This result was supported by increase in number of trabecular bone, osteoblast, osteocyte and blood vessels compared to control. Radiographically they showed that the ossification process started after two weeks and osseous fill after four weeks. The use of PRF reduced the healing time and brought faster bone regeneration.

YU ZHANG, STEFAN TANGL, CHRISTIAN D. HUBER et al (2012) [35] evaluated the effects of Choukroun's platelet-rich fibrin on bone regeneration in combination with xenograft, deproteinized bovine bone mineral in maxillary sinus augmentation. 11 sinuses from 10 patients with posterior maxillary bone atrophy were selected for the study. Six sinus floor elevations were grafted with a Bio-Oss and PRF mixture as a test group and as control group, five sinuses were treated with Bio-Oss alone. Clinical and radiographic examinations were performed after 1 week, 1, 3 and 6 months post-operatively. Bone biopsies were obtained from the grafted posterior maxilla after 6 months of sinus augmentation and un-decalcified ground sections were prepared. The characteristics of bone were evaluated using histological and histomorphometric analyses. Radiographically they revealed adequate amount of mineralized tissue and bone was similar in all cases. Histologically they showed similar morphological characteristics for both PRF and control groups. They concluded that PRF with deproteinized bovine bone had neither an advantage nor disadvantage after a healing period of 6 months.

RONALDO CELIO MARIANO, WILLIAN MORAIS DE MELO ET AL (2012) [36] evaluated the alveolar bone healing of PRP after impacted mandibular third molar surgery was compared with radiographs. In their study, they selected 15 patients with bilateral impacted mandibular third molar in similar positions. One side of the extracted socket was filled with PRP gel and the side of the socket was taken as control. The density of the bone was compared with radiographs using HLImage++ 97 software for 1 week, 1, 2, 3 and 6 months post operatively. Higher amount of bone density were observed in PRP group and also faster the bone formation compared to control group. The fibrin content present in the PRP gel permits the stabilized coagulation and facilitates the regeneration of osseous defect in early stage.

MARCO TATULLO, MASSIMO et al (2012) [37] evaluated the use of PRF in the reconstructive surgery of atrophied maxillary bones. PRF was able to regulate the inflammation and stimulate the immune process of chemotaxis. It is an autologous grafting material that eliminates any risk of disease transmission and also accelerated the new bone formation. The autologous bone graft was the only material that has the osteogenic properties apart from osteoinductive and osteoconductive properties. The biocompatibility of the grafting material depends on its preparation, eliminating the protein and lipidic components from the original material and making it inorganic before being sterilized by heat and irradiation. The deproteinized bovine bone graft (Bio-Oss) was largely used in sinus lift procedures and it was able to cause a physiological process of peri-implant bone reshaping with neoapposition and significant bone formation. The histological studies showed that an equal bone growth and trabecular organization occurred between the areas treated with PRF and the control side (F.D.B.A.). The authors concluded that with the use of PRF the healing time is significantly reduced and the implant can be placed 4 months (120 days) after surgery. After 4 months histological study revealed that bone quality between the areas treated with PRF with FDBA and the control areas with FDBA were the same.

MAJID ESHGHPOUR, MOHAMAD REZA MAJIDI et al (2012) [11] reported and discussed in their paper about periorbital skin avulsion of size 2 x 3 cm treated with PRF membrane in a 24-year old man who suffered a motorcycle accident. Uneven healing with a minimum amount of scar tissue formation was seen in the periorbital area after 8 weeks of PRF placement. They concluded that fibrin adhesives are often used in cardiothoracic and vascular surgery to seal diffuse microvascular bleeding, as well as to seal wound borders and facilitate cutaneous reuse in general and plastic surgery and to reduce postoperative hematoma. PRF is an effective

biomaterial which will enhance the post-surgical healing process and reduce the duration of the healing period.

VEENA KALBURGI, SHIVARAJ WARAD et al (2012) [10] conducted a study on the application of Platelet Rich Fibrin and osseomold bone graft in 2 different cases of intrabony defects in chronic periodontitis subjects by clinically and radiographically. Among these 2 subjects, case-1 had 2-wall defect and case-2 patient had 3-wall defect. Both the subjects came with a complaint of food impaction and with clinically accessible >7-8mm pocket. Pocket depth assessed at 6 and 9 months after the periodontal surgery revealed reduction in PPD (from 9 mm to 6 mm) and PAL (from 8 mm to 5 mm) in case 1 and in case 2 PPD (8mm to 5mm) and PAL (7mm-4mm) respectively, with appreciable radiographic bone formation in the periodontal intrabony defect. Radiographically, significant bone formation in the intrabony defect, supporting the role of various growth factors present in the PRF in accelerating the soft and hard tissue healing was appreciated. Presence of a 3-wall IBD provided the best spatial relationship for defect bridging by vascular and cellular elements from the periodontal ligament and adjacent osseous wall. They concluded that PRF is found to be clinically effective and economical than any other available regenerative materials including PRP.

SASHA JANKOVIC et al (2012) [38] conducted a 6-months randomized clinical study to compare the results achieved by the use of a platelet-rich fibrin (PRF) membrane with Connective tissue graft (CTG) in the treatment of gingival recession. All 15 patients in their study had undergone bilateral surgical treatment for gingival recession. On one side, the gingival recession was treated with CAF and PRF membrane and on the other side it was treated with CAF and CTG. There are three crucial factors for healing and soft tissue maturation: angiogenesis, growth factors and mesenchymal stem cells. The growth factor present within

platelet concentrate entrapped in the fibrin mesh up-regulate cellular activity and promote periodontal regeneration. PDGF and TGF-β are essential for tissue preparation and regeneration and it stimulates fibroblastic and osteoblastic proliferation, but suppresses epithelial cell proliferation. During centrifugation in PRF processing, platelets are activated and their massive degranulation implies cytokine release. PRF intimates cytokines, glycanic chains and structural glycoprotein enmeshed within slow polymerized fibrin network. It increases the life span of these cytokines. The healing index showed improvement in early wound healing in PRF group compared to control group. High density of fibrin fibers provides additional stability to wound and promotes rapid neoangiogenesis. The use of PRF membrane provide acceptable clinical results, enhanced wound healing and decreased subjective patient discomfort compared to CTG-treated gingival recession.

VOLKER GASSLING, NICOLAI PURCZ et al (2013) [39] evaluated the effect of PRF on bone regeneration by comparing two absorbable membranes at lateral osteotomy site in sinus augmentation. After placing two different absorbable membranes the formation starts from the floor and wall of maxillary sinus and completed at the lateral osteotomy site. To evaluate the quality of bone grafts, biopsy was done in the lateral wall of maxillary sinus because it is the last area to mineralize. Collagen membrane is used to close the one side lateral window of maxillary sinus. It enhances the TGF-β and alkaline phosphatase to promote the bone regeneration. The other side of the lateral wall was covered by PRF membrane based on natural involvement of fibrin in wound healing. The ability of PRF is to polymerize and form three dimensional supramolecular assemblies with entrapped platelet cytokines. These cytokines have mitogenic properties and mediate the chemotaxis of undifferentiated multipotent mesenchymal stem cells.

They concluded that two different absorbable membrane placed at lateral osteotomy site in sinus augmentation shows similar amount of vital bone formation.

DONALD R. HOAGLIN AND GARY K. LINES et al (2013) [40] reviewed the effectiveness of platelet rich fibrin (PRF) in the prevention of localized osteitis following lower third-molar removal bilaterally. They compared 2 groups who underwent bilateral removal of indicated mandibular wisdom teeth. One group did not receive PRF placement & other received PRF placement. The platelets contain alpha granules which release cytokines to stimulate cell migration and to enhance cellular level events to expedite wound healing. These cytokines have been well described and include the following: TGFβ-1 (transforming growth factor-beta) can stimulate osseous cellular activity, PDGF (platelet-derived growth factor) regulates the migration and proliferation of mesenchymal cells in the vicinity of the extraction site to stimulate osseous, endothelial and fibroblastic proliferation, VGEF (vascular endothelial growth factor) and EGF (epithelial growth factor) aid in the proliferation and differentiation of numerous cell types. All patients were re-evaluated for localized osteitis 7—10 days of the surgery. The incidence of localized osteitis (LO) in PRF group was 1%, and the control group showed 9.5% incidence of localized osteitis. The control group also required 6.5 hours of additional clinical time to manage LO than the study group who received PRF. From this study they demonstrated that preventive treatment of localized osteitis can be accomplished using PRF and it enhanced the third-molar socket healing/clot retention. It greatly decreased the clinical time required for postoperative management of LO.

S. GIRISH RAO et al (2013) [8] evaluated the effects of bone regeneration on autologous platelet- rich fibrin gel in the extracted sockets. Bilateral transalveolar third molar extraction was done on 22 patients. On one side PRF gel were placed and the other side was taken as control.

The bone regeneration was measured using serial radiographs (RVG) at immediate 1, 3 and 6 months post-operatively. Higher mean pixels was recorded in case side compared to control side at immediate post op, 1 month post op, 3 months post op and 6 months post op. However, the difference in the mean pixels recorded between the two groups was not statistically significant. In their study, they indicated that there was a definite improvement in the regeneration of bone after third molar surgery treated with PRF as compared to the control group. PRF increases the bone density and accelerates the hard tissue regeneration.

BAJAJ P, PRADEEP AR, AGARWAL E, RAO NS et al (2013) [41] aimed to explore the clinical and radiographical effectiveness of autologous platelet-rich fibrin (PRF) and autologous platelet rich plasma (PRP) in the treatment of mandibular degree II furcation defects in chronic periodontitis. In their study mandibular degree II furcation defects were treated with either autologous PRF with open flap debridement or autologous PRP with OFD or OFD alone in 72 patients. They assessed the probing depth, relative vertical clinical attachment level and horizontal clinical attachment level along with gingival marginal level by clinically and radiologically. They found that all clinical and radiographic parameters showed significant improvement on both the test sites compared to those with OFD alone. Relative vertical and horizontal clinical attachment level gain was also greater in PRF and PRP sites as compared to control site. They concluded that use of autologous PRF and PRP were effective in the treatment of furcation defects with uneventful healing of sites. There was no significant difference between the use of PRF and PRP in the furcation defects. The treatment with autologous PRF and PRP stimulated a significant reduction in PD and increase in the Relative Vertical Clinical Attachment Level and Relative Horizontal Attachment Level gain and bone filled when compared to OFD at 9 months post operatively.

GUILHERME DE MARCO ANTONELLO et al (2013) [42] assessed the effects of PRP on new bone formation in the extracted socket clinically and radiographically for about 6 months. They took a sample of 25 patients with extraction of all four impacted third molars with similar orientation, depth, and root morphology. After extraction PRP was placed in one of the extracted socket whereas the other side taken as (control). Patients were under clinical and radiographic follow-up for 6 months. Bone repair was assessed by Periapical radiographs taken immediately after extraction and at 1, 3, 5 months. The radiographs were analysed with image tool software and the density was measured using histogram analysis. They showed that PRP treated and nontreated socket are alike but greater difference were detected in maxilla compare to mandible due to structural organization of bone and richer blood supply thus allows faster healing. They concluded that PRP provides a safe and effective means of speeding alveolar bone repair.

PRISANA PRIPATNANONT, THONGCHAI NUNTANARANONT et al (2013) [43] investigated the effect of platelet-rich fibrin (PRF) on bone regeneration of various grafting materials (autogenous graft and deproteinized bovine bone) in rabbit calvarial defects. DBB and calvarial bone chips were derived from heat treatment at 1200°c with grain size 0.25-1mm. They choose 20 New Zealand white rabbits with two bicortical skull defects and 10 rabbits were treated with PRF and other 10 with non-PRF. In 5 animals one side of the defect was empty and paired with an autologous bone graft on other side. In other 5 animals one side of the defect was filled with autologous bone and DBB and paired with DBB alone on other side. Densitometry and histomorphometric analysis were done for measuring bone formation. In mean optical density (OD) and histomorphometric analysis (HA) the percentage of new bone formation in the PRF group was significantly higher than the non-PRF group. No significant difference in deproteinized bone graft alone defect. They concluded that PRF had a positive effect on bone

formation when used alone or combined with autogenous bone, but not with deproteinized bovine bone.

BALARAM NAIK, PKARUNAKAR, M JAYADEV et al (2013) [3] reviewed and discussed the role of PRF as a wound healing aid in dentistry. They searched scientific papers published upto March 15, 2013 with a custom range of 5 years and they selected 72 papers with all inclusion and exclusion criteria. They revealed that PRF was used to promote wound healing, bone regeneration, graft stabilization, wound sealing and hemostasis. Because the fibrin matrix is better organized, it is able to more efficiently direct stem cell migration and promote healing program. The slow polymerization mode confers to PRF membrane as a particularly favourable physiologic architecture to support the healing process.

M. MARRELLI, M. TATULLO (2013) [44] assessed the healing of bone and soft tissue around post extraction dental implant using PRF. In their study, they placed implants in the immediate post extraction site on upper maxillary bone for 59 patients. After placing implants the surgical site were covered with PRF membrane between the alveolar crest and implant. They evaluated the peri-implant responses by clinically, radiologically, histologically and suggested that proper immediate post extractive implant placement is followed by supracrestal biological width formation along with the abutment. They revealed that immediate implants will shorten the completion of rehabilitation while reducing bone reabsorption of the residual alveolus and thus avoiding the need for second surgical intervention. They concluded that PRF helps to achieve preservation of tooth like tissue contours and formation of mature bone tissue around the implants.

SUTTAPREYASRI SRISURANG, BUASOD KANTHEERA, et al (2014) [45] evaluated the potential of platelet-rich fibrin (PRF) and epithelialized palatal free graft (FGG) for preserving

the alveolar ridge after tooth extraction in minipigs, by clinically and radiographically. They assigned four groups. Group I – alveoli filled with PRF, group II- alveoli sealed with FGG and group III – alveoli filled with PRF and sealed with FGG and group IV – alveoli filled with a blood clot and allowed to heal spontaneously (control). During 1st week all alveoli healed uneventfully and no infection was observed. After 2 weeks, the granulation tissue covering the socket was denser and more mature in PRF group when compared with other group but it was not covered completely by epithelium. After 6 weeks all sites were covered with matured epithelium. After 12 weeks, the alveolar ridge resorption was seen in bucco-lingual and occluso-gingival direction in all the groups. The bone height was decreased in 6 weeks after extraction but it gradually increased until 12 weeks. But in FGG the bone height was further decreased from 6 to 12 weeks. In PRF group only the bone height was increased compared to other groups radiographically. Histomorphometrically, the newly formed bone in PRF group was increased from 2nd to 12th weeks, when compared to others. They concluded that PRF promotes faster soft and hard tissue healing of extracted socket in first 2 weeks and in 12 weeks PRF enhances bone healing and preserves the marginal bone height and width.

JOY R. DAS, P. SREEJITH VALIYA PARAMBATH et al (2014) [(7)] aimed to improve the hard and soft tissue healing using platelet rich plasma after mandibular third molar surgery. They evaluated the bone density, soft tissue healing, post-operative pain, swelling and alveolar osteitis 1st, 2nd, 7th day, 1st and 2nd month postoperatively. 12 patients were selected for their study. They underwent bilateral surgical removal of mandibular impacted third molars. After removal of the impacted tooth one of the randomly selected socket was filled with PRP gel and the other side was taken as control. The pain was assessed by VAS scale and the bone density was measured using OPG by grey scale value using adobe photoshop software. They revealed that the PRP

significantly showed greater volume of new bone formation compared to control side and there was reduction of pain in the PRP side. The soft tissue healing, swelling, and incidence of dry socket showed statistically insignificant values when compared to PRP and non-PRP.

B SARAVANA KUMAR, A. JULIUS et al (2014) [46] highlighted the clinical use of PRF following extraction and PRF protocol in oral and maxillofacial surgery for improving bone healing in implant dentistry. The use of PRF as the sole filling material during a simultaneous sinus lift, stabilized a high volume of natural regenerated bone in the subsinus cavity up to the tip of the implants. PRF membranes protect the surgical site and promote soft healing in implant tissue healing and cut few millimeters PRF fragments mixed with graft material functioned as a "biological connector" between the different graft elements and as a matrix that supports neo-angiogenesis, capture of stem cells and migration of osteoprogenitor cells to the center of the graft. The biologic activity of fibrin molecule has cicatricial capacity and the slow polymerization mode confers to the PRF membrane a particularly favorable physiologic architecture to support the healing process.

MOHAMED H.EL KENAWY, UNA M.EL SHINNAWI et al (2014) [47] evaluated the clinical radiographic efficacy of PRF membrane used with immediately placed implants. The implants were placed immediately after extraction of teeth, positioned 2-3 mm beyond the apex to achieve more primary stability. The gap between the socket and implant thread was grafted with DBBM. The PRF enhances the clot and graft stability. The platelets release cytokines such as PDGF, IGF, and VEGF. PDGF and IGF stimulate osteogenic cytokines and the growth factor release for about 7 days. PRF allows to avoid the use of membranes and barriers. It reduces the risk of exposure to the oral cavity and reduces the bacterial contamination. Implants were successfully osseointegrated over one year follow up period with insignificant change in crestal bone level.

LOKMAN ONUR UYANIK1, KANI BILGINAYLAR1 AND İLKER ETIKAN (2015) [48] aimed to compare the post-operative outcomes in impacted mandibular third molars that were treated using either platelet-rich fibrin (PRF), a combination of PRF and piezosurgery, or conventional rotatory osteotomy. Bilaterally symmetrical impacted lower third molar were removed in 20 patients. Patients were divided into two main groups. In group A, traditional surgery was performed on one side, traditional surgery was performed and PRF was administered to the extracted socket on the other side. In group B, on one side, piezosurgery was used for osteotomy, traditional surgery was performed and PRF was administered on the other side of same patient. They assessed pain, trismus and swelling at 1, 2, 3, and 7 days postoperatively. They concluded that in their study, the combined use of PRF and piezosurgery significantly decreased the number of analgesics taken and it reduces the pain, swelling and trismus after surgery.

MUSTAFA TUNALI, HAKAN et al (2015) [49] introduced Titanium-prepared, platelet-rich fibrin (T-PRF) a new platelet concentrate. In this method of preparation, titanium tubes were used and were more effective in activating platelets than the glass tubes. This material is used to avoid any adverse effects in the dry glass or glass-coated plastic tubes i.e silica. They found that titanium- induced platelet aggregation and clot formation was similar to glass tubes but the only difference is that it increases the biocompatibility of T-PRF. T-PRF is a new platelet product that induced formation of new connective tissue and resulted in an excellent bone regeneration within 15 days.

PAVAN KUMAR, VIKRAM REDDY et al (2015) [50] discussed the disadvantages of using glass tube for centrifugation and developed advance methods of PRF preparation. Problem with the glass-evacuated collection tubes is the possibility of health hazard from silica activators.

Small fraction of the glass tube's silica particles remain suspended in the fibrin and platelet-poor layers of plasma, which might reach the patient when PRF is used for treatment. Titanium tubes were used in preparation of PRF. They were termed T-PRF. T-PRF was more woven and thick polymerized fibrin when compared with L- PRF and it is due to the biocompatibility and hemocompatibility of titanium. The centrifugation protocol of T-PRF is 2800rpm for 12mins.

MICHEL KNAPEN, DAMIEN GHELDOF, PIERRE DRION, et al (2015) [51] conducted a study to compare the potential effect of L -PRF on osteogenesis in a standardized model. 72 hemispheres were implanted on the calvaria of 18 rabbits and on each rabbit four different study materials were placed: L- PRF, bovine hydroxyapatite (BHA), BHA with L-PRF and an empty hemisphere was used as control. Histological and histomorphometrical analyses were done. They showed that osteogenis formation was adequate in all four groups and no significant difference was seen in bone regeneration in L-PRF alone or combined with BHA. At the time sugery they placed L-PRF very placed in the calvaria lead to lack of positive effect on quality, kinetics and bone regeneration quantity. It also stimulates angiogenesis and fibroblast migration. L-PRF did not have any positive effect on connective cell proliferation because it blocks the cell penetration due to the high density of L-PRF.

V. MORASCHINI, BARBOZA et al (2015) [6] evaluated the effects of autologous platelet concentrates in the preservation of extracted socket. The plasma concentrates rich in growth factors stimulate the soft and hard tissue repair and regeneration. It also reduces the inflammation, pain and discomfort. The method used to prepare plasma concentrates may affect plasma quality directly and indirectly. The speed, time and the type of anti-coagulants used in centrifugation may affect the quality and quantity of cells in PRP, because the difference between the natural clotting process after centrifugation and the addition of anticoagulating

agents such as thrombin, and calcium hydrochloride will change the biochemical properties of blood. After placing the platelet concentrates in the extraction socket sequence of healing will occur. At first week after extraction the primary clot undergoes remodelling which initiates the formation of the osteoid matrix. After 38 days, the socket is filled with two-thirds of trabecular bone and after 10 weeks the socket is completely filled with bone at 10 weeks after extraction. The healing time of extraction sockets varies significantly between individuals. Remodelling from woven bone to lamellar bone occurs slowly. In the phases of healing and epithelialization of soft tissues plasma concentrate cells tend to populate the surgical wound with growth factors such as PDGF, TGF-b, and VEGF topically placed inside the socket and tend to accelerate soft tissue healing by stimulating angiogenesis, granulation tissue formation and epithelialization. It not only improves the healing but also show positive gains in keratinized gingiva after soft tissue surgeries using PRF when compared with spontaneous healing in control groups.

TEJESH YELAMALI, D.SAIKRISHNA et al (2015) [52] evaluated and compared the effect of PRF with PRP on soft and hard tissue healing of third molar extracted sockets. Platelet plays a role in hemostasis and is a natural source of growth factors. These include PDGF, insulin like growth factor (IGF), platelet derived angiogenic factor (PDAF) and VEGF. PRP is a fibrin framework of platelets. It has the potential to support regenerative matrix. Fibrin is the activated form of a plasmatic molecule called fibrinogen. In fact, fibrinogen is the final substrate of all coagulation reactions. Being a soluble protein, fibrinogen is transformed into an insoluble fibrin by thrombin while the polymerized fibrin gel constitutes the first cicatricial matrix of the injured site. PRF is considered as a fibrin biomaterial. Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts. It permits rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue.

Therefore, these PRF membranes can be used for all types of superficial cutaneous and mucous healing. Moreover with PRF, a progressive polymerization mode signifies the increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines). Such a configuration implies an increased lifespan for these cytokines, because it will be released and used only at the time of initial cicatricial matrix remodeling (long-term effect). These cytokines are available for a convenient period, only when the cells start cicatricial matrix remodelling. The PRF is more effective than PRP because the PRF clot forms a strong fibrin matrix with a complex 3-dimensional architecture. It does not dissolve quickly instead the strong fibrin matrix is remodeled slowly to the form a natural blood clot. Thus the growth factors can keep their activity for a relatively longer period and stimulate the bone regeneration effectively.

MATERIALS & METHODS

SOURCE OF DATA:

This clinical study was undertaken on outpatients, as minor oral surgical procedure in the Department of Oral and Maxillofacial Surgery, Sri Ramakrishna Dental College and Hospital, who required prophylactic surgical removal of bilateral impacted mandibular third molars. The study confirmed to the ethical guidelines and was approved by the institutional Ethical committee.

METHODOLOGY:

A randomized clinical trial was conducted in which patients satisfying the inclusion criteria, as listed below, were assigned for surgical removal of bilateral impacted lower third molars.

Surgery was done by the same operator on both the sides of the same patient. Different patients were handled by different surgeons.

Surgery was performed on the test side first and PRF placed followed by surgery on control side.

Inclusion criteria:

1. Patients were aged between 18-35 years.
2. Patients who required prophylactic surgical removal of bilateral impacted mandibular 3rd molar bilaterally.
3. Patients with no general medical contra indication for oral surgical procedures. (American Society of Anesthesiologists [ASA]-I or ASA-2 rating).

Exclusion criteria:

1. Cysts, dental caries or pericoronitis present in relation to third molar tooth.
2. An active infection or a history of persistent infections.
3. Presence of uncontrolled diabetes mellitus, immune diseases or other systemic conditions contraindicating the procedure.

STUDY POPULATION:

According to the criteria enumerated above, twenty five patients (Annexure 1) were recruited (both women and men). Each patient was given oral explanation and written information stating about the surgical procedure and possible discomforts, risks and complications. Patients were informed of their participation in a randomized trial. Written consent was obtained from the patients /guardians who participated in the study (Annexure 4). Each of the twenty five patients were subjected to bilateral surgical removal of impacted mandibular third molar on the same visit, performed by the same surgeon on both the sides.

Each patient was categorized into two groups:

1. PRF / Case side: surgical removal of third molar and placement of Autologous Platelet Rich Fibrin (PRF) in socket followed by primary closure of the socket.
2. Control side: surgical removal of third molar followed by primary closure of the socket. The side chosen as case side/ control side was random.

Presurgical Evaluation: A complete clinical examination and investigation (Annexure 4) with orthopantomogram were carried out prior to the surgery.

WAR lines assessment and Pederson's Difficulty Index were performed using radiographs as a part of treatment planning.

PREPARATION OF PLATELET RICH FIBRIN (PRF): The PRF was prepared in accordance with the protocol developed by Choukroun et al [(14)]. Under sterile aseptic conditions about 20ml of venous blood was taken from the patient (fig.3). Blood was transferred to sterile 15ml test tubes immediately without adding any anticoagulant. They were centrifuged at 3000 rpm for 10 minutes (fig 2).

After the processing of venous blood in the centrifugal machine, 3 distinct layers will be seen as follows (fig 4):

1. The supernatant layer representing acellular plasma or PPP.
2. Intermediate layer, consisting of the exudate resulting from PRF clot corresponding to the platelets trapped in the fibrin meshes.
3. Red blood corpuscles at the bottom of the test tube.

The gel of PRF formed is separated with the intermediate buffy coat from the RBC's using scissors (fig 5&6).

The PRF gel was placed between two sterile gauzes and squeezed to make it into a membrane (fig 7&8).

- Platelet rich fibrin (PRF) obtained is used in the extraction socket after the surgical removal of the third molars.

ARMAMENTARIUM:

In general, the armamentarium employed for carrying out the surgical procedure is as follows (fig 1):

- Mouth Mirror
- Shepherd's crook explorer
- William's Probe
- 20 ml syringe
- 15 ml test tube with lid
- Test tube holder
- Test tube stand
- 26 gauge needle and 2cc syringe

- Lignocaine hydrochloride 2% with adrenaline bitartrate 1 :80000
- Bard Parker handle no.3
- No. 15 Surgical blades
- Sterile gauze
- Bite block
- Molt's NO. 9 Periosteal elevator and Howarth's Periosteal elevator
- Austin's Retractor
- Micromotor and High speed Straight Rotory Handpiece
- Bone cutting burs [round bur and straight fissure bur no.701/ 702/ 703]
- Coupland elevator
- Cryer's elevator
- Halstead's Mosquito Artery forceps
- Lucas Curette
- Needle holder
- Adams tissue holding forceps- Toothed and Non-toothed
- Stitch Scissors

SURGICAL PROCEDURE:

1. Local Anaesthesia was achieved on the test side by conventional Direct technique of Inferior Alveolar Nerve Block using 2% Lignocaine Hydrochloride with Adrenalin bitartrate in the concentration of 1:80,000.
2. Modified Ward's incision placed and a mucoperiosteal flap was reflected to expose the tooth and bone with Molt's No.9 Periosteal elevator.

3. Bone guttering was done along buccal and distal aspect of tooth using a 702 straight fissure bur at a rotary speed of 35,000 Rotation per minute under constant copious saline irrigation.
4. Tooth was sectioned according to the necessity and was delivered using Coupland elevator.
5. The tooth follicle remnants attached to the socket if any were curetted by Lucas curette.
6. Remnants of bone were removed and sharp bony edges were smoothened with a bone file.
7. Socket thoroughly debrided with betadine and saline to clear of debris (fig 9).
8. Platelet rich fibrin (PRF) obtained as mentioned above is placed using Adams tissue holding non toothed forceps in one of the extracted socket (fig10&11).
9. Reflected flap were re-approximated to their original position and primary closure achieved by suturing with non-absorbable 3-0 black braided silk (fig 12).
10. Same procedures was carried out on control side from steps 1-9 except step 8

Post-operative care:

Antibiotic cover:

- Cap. Amoxicillin 500mg thrice daily for 3 days.
- Tab. Metronidazole 400mg thrice daily for 3 days.

Analgesics were prescribed:

- Tab. Aceclofenac 100 mg + Paracetamol 325mg b.i.d for 3 days.
- Extra-oral ice pack advised on both operated sides for 3-4 hrs postoperatively.
- Mouth opening exercise advised for 1 week
- Sutures were removed one week postoperatively.

RADIOGRAPHIC EVALUATION:

Orthopantomogram was taken prior to surgery in ORTHOPHOS XG5 machine using sidexis software. Similar orthopantomagrams were taken 1 month, 3 months and 6 months after surgery. The radiodensity of both the sockets were measured using the Matlab Software.

Image processing tool box from Matlab software is used in many applications for processing and analyzing the images. It also facilitates development of user-defined algorithms.

We used this tool box to measure the density of PRF and Non-PRF regions.

Algorithm developed in Matlab to measure the density from an OPG image is as follows:

1. OPG image taken from the patient is input into the image processing tool box,
2. OPG image which is of Red-Green-Blue (RGB) format is converted to gray scale (Black/White) image,
3. Pixel values from PRF region and Non-PRF region are separately extracted.
4. Average of the extracted pixel values from PRF region is computed.
5. Similarly average of the extracted pixel values from Non-PRF region is computed.
6. Each pixel value obtained conveys the intensity of that region. Intensity value ranges between 0 and 255, with 255 indicating the densest region.

Hence average of the pixel value conveys the density over that region.

Later, the density values of PRF region and Non-PRF region in 1^{st}, 3^{rd} and 6^{th} month were compared in order to see the improvement in bone healing following placement of PRF in the extraction socket.

FIGURES

Figure 1- Armamentarium

Figure 2- Centrifuge Machine

Figure 3 – Venous Blood withdrawn from patient

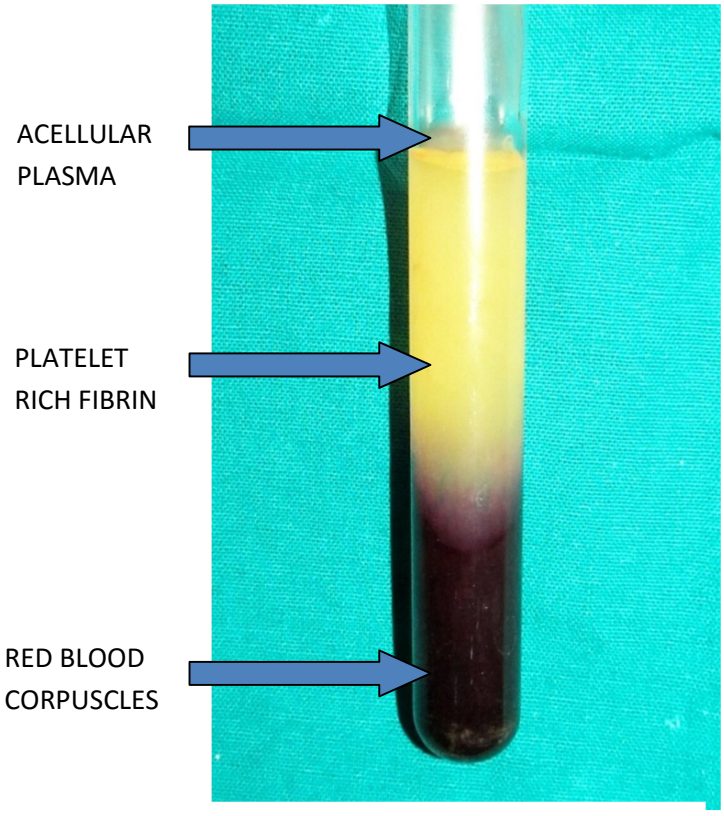


Figure 4 – after centrifugation

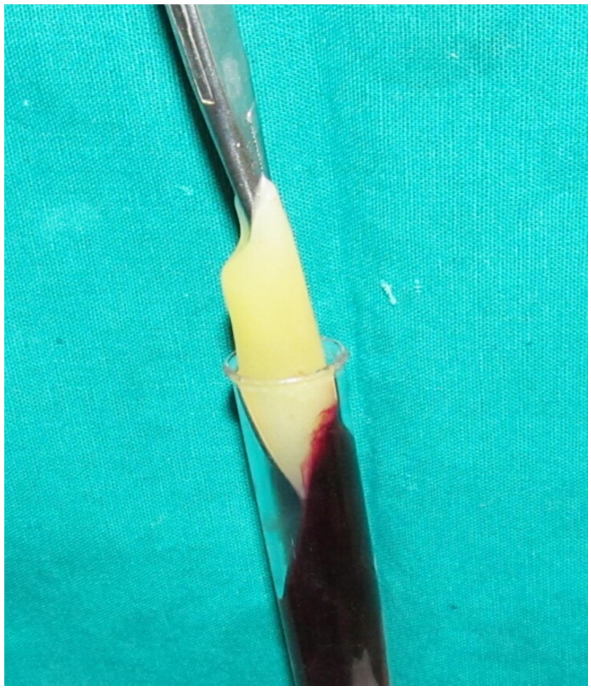


Figure 5 -PRF taken using Hemostat

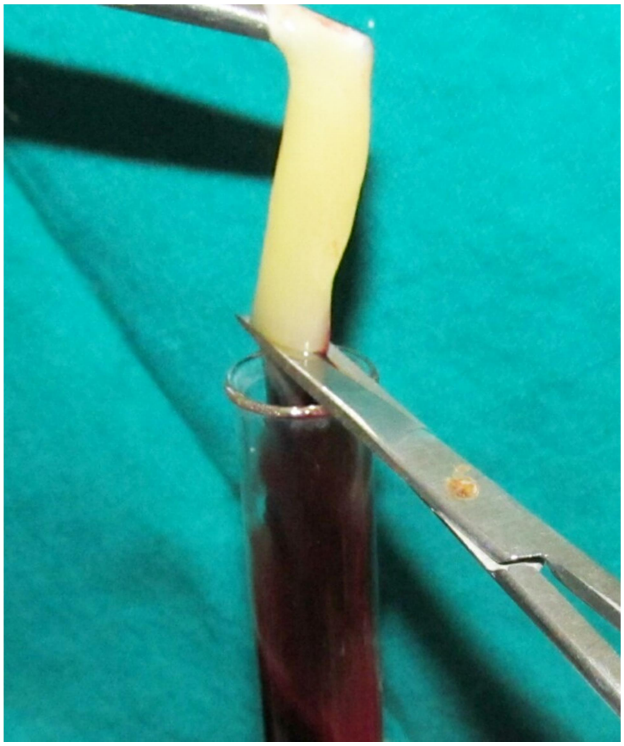


Figure 6 - separating PRF clot using scissors

Figure 7- PRF

Figure 8 - Squeezed PRF membrane

Figure 9 - Extraction socket

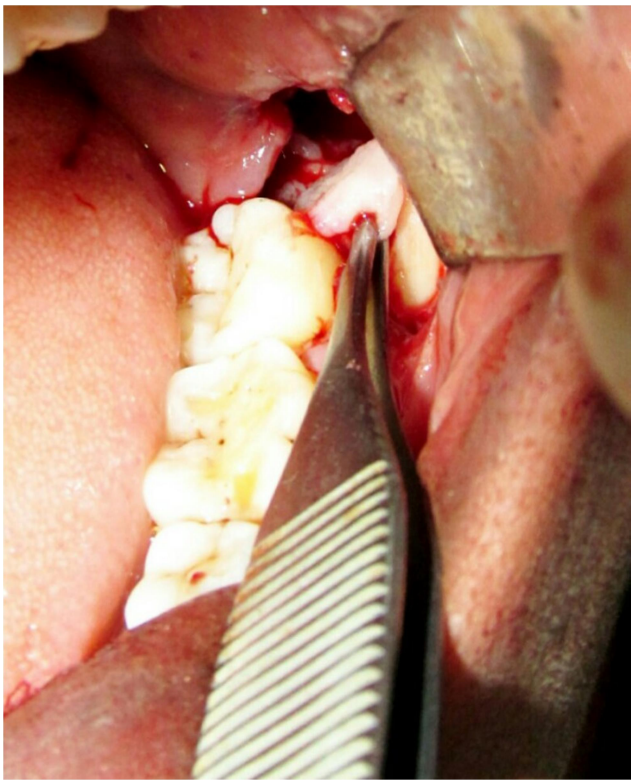


Figure 10 - Placing PRF in the socket

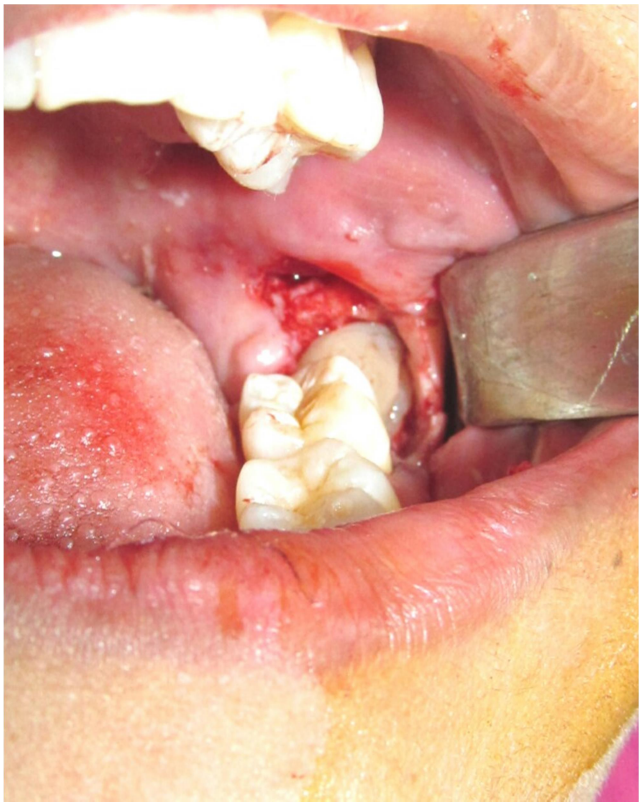


Figure 11- PRF membrane

Figure 12- Primary closure

STUDY DEMONSTRATION

A 21 year old male Patient named Mr. Joshua came to our Department of Oral and Maxillofacial Surgery, Sri Ramakrishna Dental College and Hospital, with the chief complaint of cheek bite for past 1 week on both the sides. He did not have any relevant medical history. On intra oral examination, 38, 48 and 18 were partially erupted. Radiographic investigation was done and the OPG revealed mesioangularly impacted 48 and horizontally impacted 38. The treatment plan was surgical removal of 38 and 48 under LA in a single session with PRF placement in extraction socket of 38. Informed consent was obtained. Patient was taken to biochemistry laboratory and PRF was prepared using our protocol. Sterile aseptic precautions were undertaken. Bilateral Inferior Alveolar Nerve Block was given. Surgical removal of 38 performed, PRF was placed in the socket and primary closure was done, followed by surgical removal of 48 with primary closure. Patient was recalled after two days for review. Patient had mild swelling on right side and no swelling on left side. Patient was recalled after 1 week for suture removal. Post-operative OPGs were taken after 1 month, 3 month and 6 month. The 1st, 3rd and 6th month follow-up radiograph showed significant bone regeneration in PRF region compared to non-PRF region.

SRI RAMAKRISHNA DENTAL COLLEGE & HOSPITAL

SNR COLLEGE ROAD, COIMBATORE- 641006.

CASE RECORD

PATIENT OP NO: 1728 STUDY NO: 5

NAME: Mr. Joshva AGE/SEX: 21/M

ADDRESS: Dindigal. OCCUPATION: Student

CHIEF COMPLAINT:

Pt c/o of irregularly arranged teeth

VITAL SIGN :

PULSE	72 beats/min
BP	110/80 mmHg
TEMPERATURE	Afebrile
RESPIRATORY RATE	16 breath/min

HISTORY OF PRESENTING ILLNESS:

H/o irregularly arranged tooth and no associate symptom of pain.

PAST MEDICAL HISTORY:

- NRMH -

PAST DENTAL HISTORY:

Pt. undergone restoration before 3months and undergone extraction before 6yrs.

PERSONAL HISTORY:

Brushes once daily with paste and brush.

GENERAL EXAMINATION

GAIT: Normal BUILT: well built

LOCAL EXAMINATION:

EXTRA ORAL EXAMINATION

FACIAL ASYMMETRY : Apparently symmetrical

TMJ EXAMINATION : No pain, clicking sound, deviation.

LYMPH NODES : No palpable lymph nodes

INTRA ORAL EXAMINATION :

MOUTH OPENING : 45mm

SOFT TISSUES :

- Normal -

HARD TISSUE : No. of teeth present $\frac{8\,7\,6\,5\,4\,3\,2\,1 \mid 1\,2\,3\,4\,5\,6\,7\,8}{7\,6\,5\,4\,3\,2\,1 \mid 1\,2\,3\,4\,5\,6\,7}$

PERIODONTAL STATUS:

38 → Mesio Buccal - 2
Mesio Lingual - 3

48 → Mesio Buccal - 3
Mesio Lingual - 5

STATUS OF THIRD MOLAR :

38 - partially erupted

48 - partially erupted.

INVESTIGATION :

IOPA

PANTOMOGRAM

DIAGNOSIS :

Mesio angular Impaction - 48

Horizondal Impaction - 38

TREATMENT PLAN :

Adv. surgical removal of 38, 48.

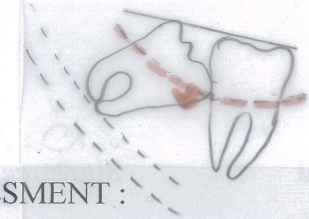
III MOLAR ASSESSMENT

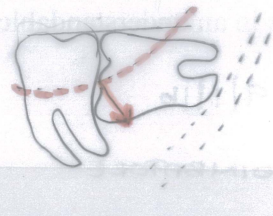
PELL & GREGORY'S CLASSIFICATION:

	R	L
CLASS	II	II
POSITION	A	A
ANGULATION	Mesioangular	Horizondal

WAR LINES:

RIGHT: LEFT:





WHARFE's ASSESMENT :

	R	L
WINTER'sCLASSIFICATION	1	2
HEIGHT	0	0
ANGULATION	1	4
ROOT SHAPE	1	1
FOLLICLE	0	0
EXIT PATH	2	2
TOTAL SCORE	5	9

PEDERSON'S DIFFICULTY INDEX:

RIGHT:

Mesioangular - 1
Class II - 2
position A - 1
4 Minimally difficult

LEFT:

Horizondal - 2
class II - 2
position A - 1
5 Moderately difficult

TREATMENT DONE:

PROCEDURE :

RIGHT: Ward's incision placed. Buccal bone guttering done. Tooth removed by mesial point of application.

LEFT: Ward's incision placed. Buccal bone guttering done. Tooth splits into mesial & distal part.

COMPLICATIONS IF ANY: RIGHT: Lingual cortical plate # — LEFT: -

DURATION : RIGHT: 20 mins — LEFT: 30 mins

MEDICATIONS:

℞
- cap Amoxycillin (500mg) 1-1-1 (5) x 3days
- Tab Metronidazole (400mg) 1-1-1 (5) x 3days
- Tab Aceclofenac (100mg) + Paracetamol (500mg) 1-0-1 (6) x 3days
- Tab pantoprazole (20mg) 1-0-1 (6) x 3days

29.1.16

Signature of the operator

INFORMED CONSENT FORM

I JOSHUVA (name of the patient) hereby authorize the doctor in charge to administer treatment and to perform any such procedure as may be deemed necessary or advisible in diagnosis and treatment. I have been explained about my oral conditions and the treatment planned.

I hereby give full consent to undergo surgical removal of my impacted tooth under local anesthesia and placement of Autologous Platelet Rich Fibrin from my own blood in one of the extracted socket. The doctor has explained to me about the advantages, disadvantages and the risk associated with the procedure in an understandable language.

Date: 29/1/16

Place: COIMBATORE

R. Joshuva
Signature of the patient

N. Saravanan
Signature of the doctor

POST OPERATIVE REVIEW:

	RIGHT	LEFT	SIGNATURE
2nd DAY	Diffuse mild Swelling present	Diffuse mild Swelling present	1/2/16
1 WEEK	-Nil-	- Nil -	
1st MONTH	Radiolucency & Radioopacity present in the socket	Radiolucency & Radioopacity present in the socket.	
3rd MONTH	Radiolucency & Radioopacity present in the socket.	Radiolucency & radioopacity present in the Socket	
6th MONTH	Radiolucency & Radioopacity present in the socket.	Radiolucency & radioopacity present in the socket.	

Figure 13 - Mr. Joshua

Figure 14 - Intra oral Pre-operative view

Figure 15 - Pre-operative OPG

Figure 16 – Extraction Socket

Figure 17 – After placing PRF in the socket

Figure 18 – Primary closure

Figure 19 – Post operative following on suture removal

Figure 20 – 1 month post operative OPG

Figure 21 – 3 month post operative OPG

Figure 22 – 6 month post operative OPG

RESULTS

In our study, out of twenty five patients who were operated five patients did not turn up for the 3rd and 6th month follow-up and hence they were excluded from the study. Out of twenty patients, two patients had a postoperative complication of paraesthesia on the control side and 1patient had paraesthesia on test side and it reduced after two to three weeks of follow up. Fifteen patients had post-operative complication of mild swelling and pain in the Non-PRF region and five patients had diffuse swelling and pain in the PRF region for two days and it subsided after three days. We did not come across any other complication like trismus, dry socket or wound dehiscence.

Patients were reviewed on 1month, 3months and 6months postoperatively, and OPG were taken during those visits. The OPGs were analyzed with Matlab software. Both the sockets were compared for the radiodensity. Results were expressed by statistical analysis: SPSS 17.0 software as mean ± standard deviation.

A p value < 0.05 (5% and 1%) was considered statistically significant. Kolmogorov-Smirnov test (Annexure 3) was applied to continuous variables to check for normality distribution. Test results showed the measurements follow normal distribution (all P>5%). Therefore, the results were statistically analyzed by parametric methods such as Repeated Measure ANOVA and paired't' test application of all and pair-wise comparison.

RADIOGRAPHIC ANALYSIS: (tables 1-8)

- Comparison of two sides with respect to radiographic scores at 1st month, 3rd month and 6th month post operatively are as follows.
- From Paired t test results, significant difference was noted on 1st month post-operative follow up (p=0.061>5%) with more radiodensity on PRF side compared to non-PRF side.

- From Paired t test results, a highly significant difference was noted on 3rd month post-operative review (p=0.000<1%) with more radiodensity on PRF side compared to non-PRF side.
- From Paired t test results, a highly significant difference was noted on 6th month post-operative OPG (p=0.000<1%) with more radiodensity on PRF side compared to non-PRF side.
- From the results of Repeated Measure ANOVA, a significant difference was noted on 1st, 3rd and 6th month of PRF side post operatively (p=0.001).
- In graphical representation there was a significant difference when comparison of 1st, 3rd, 6th month of PRF side with non-PRF side, the mean value ranges from 142.21±3.7, 153.41±2.4, 155.51±3.2 in PRF side and 130.93±4.7, 136.33±3.6, 135.29±2.8 in non-PRF side.

Statistical density comparison between PRF and non-PRF region are:

- In the 1stmonth follow-up radiograph showed significant regeneration in PRF region compared to non-PRF region for fifteen patients.
- During 3rd month follow-up radiograph, significant regeneration was observed in PRF region over non-PRF region for nineteen patients.
- At the 6th month follow-up radiograph, significant difference between PRF and non-PRF region is seen in all the twenty patients.

Table 1: Comparing PRF and non PRF using paired sample statistics

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	PRF- 1 month	142.2170	20	16.81088	3.75903
	NON PRF- 1 month	130.9305	20	21.06706	4.71074
Pair 2	PRF- 3 month	153.4175	20	10.86490	2.42946
	NON PRF- 3 month	136.3345	20	16.45848	3.68023
Pair 3	PRF- 6 month	155.5135	20	14.33338	3.20504
	NON PRF- 6 month	135.2955	20	12.55874	2.80822

Table 2: Comparing PRF and non PRF using paired sample statistics and measure the p value

Paired Samples Test									
		Paired Differences					t	df	P VALUE
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	PRF- 1 month - NON PRF- 1 month	11.28650	25.29932	5.65710	-.55395	23.12695	1.995	19	.061
Pair 2	PRF- 3 month - NON PRF- 3 month	17.08300	16.03517	3.58557	9.57831	24.58769	4.764	19	.000
Pair 3	PRF- 6 month - NON PRF- 6 month	20.21800	15.10284	3.37710	13.14965	27.28635	5.987	19	.000

Table 3: Repeated Measure ANOVA -Tests of Within-Subjects Effects

Measure:PRF						
Source		Type III Sum of Squares	df	Mean Square	F	P value
Observed Time	Sphericity Assumed	2044.276	2	1022.138	8.000	.001
	Greenhouse-Geisser	2044.276	1.345	1520.122	8.000	.005
	Huynh-Feldt	2044.276	1.410	1449.702	8.000	.004
	Lower-bound	2044.276	1.000	2044.276	8.000	.011
Error(Time)	Sphericity Assumed	4854.892	38	127.760		
	Greenhouse-Geisser	4854.892	25.551	190.005		
	Huynh-Feldt	4854.892	26.793	181.203		
	Lower-bound	4854.892	19.000	255.521		

Table 4: Repeated Measure ANOVA -Tests of Within-Subjects Effects

Measure:NON-PRF						
Source		Type III Sum of Squares	df	Mean Square	F	p-value
Time	Sphericity Assumed	328.906	2	164.453	1.606	.214
	Greenhouse-Geisser	328.906	1.857	177.079	1.606	.216
	Huynh-Feldt	328.906	2.000	164.453	1.606	.214
	Lower-bound	328.906	1.000	328.906	1.606	.220
Error(Time)	Sphericity Assumed	3890.425	38	102.380		
	Greenhouse-Geisser	3890.425	35.291	110.240		
	Huynh-Feldt	3890.425	38.000	102.380		
	Lower-bound	3890.425	19.000	204.759		

Table5: Pair wise T-Test PRF

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	PRF- 1 month	142.2170	20	16.81088	3.75903
	PRF- 3 month	153.4175	20	10.86490	2.42946
Pair 2	PRF- 1 month	142.2170	20	16.81088	3.75903
	PRF- 6 month	155.5135	20	14.33338	3.20504
Pair 3	PRF- 3 month	153.4175	20	10.86490	2.42946
	PRF- 6 month	155.5135	20	14.33338	3.20504

Table 6: Comparing PRF using paired samples test and measure p value

Paired Samples Test									
		Paired Differences					t	df	P value (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	PRF- 1 month - PRF- 3 month	-11.20050	17.37204	3.88451	-19.33086	-3.07014	-2.883	19	.010
Pair 2	PRF- 1 month - PRF- 6 month	-13.29650	19.53349	4.36782	-22.43845	-4.15455	-3.044	19	.007
Pair 3	PRF- 3 month - PRF- 6 month	-2.09600	9.12234	2.03982	-6.36539	2.17339	-1.028	19	.317

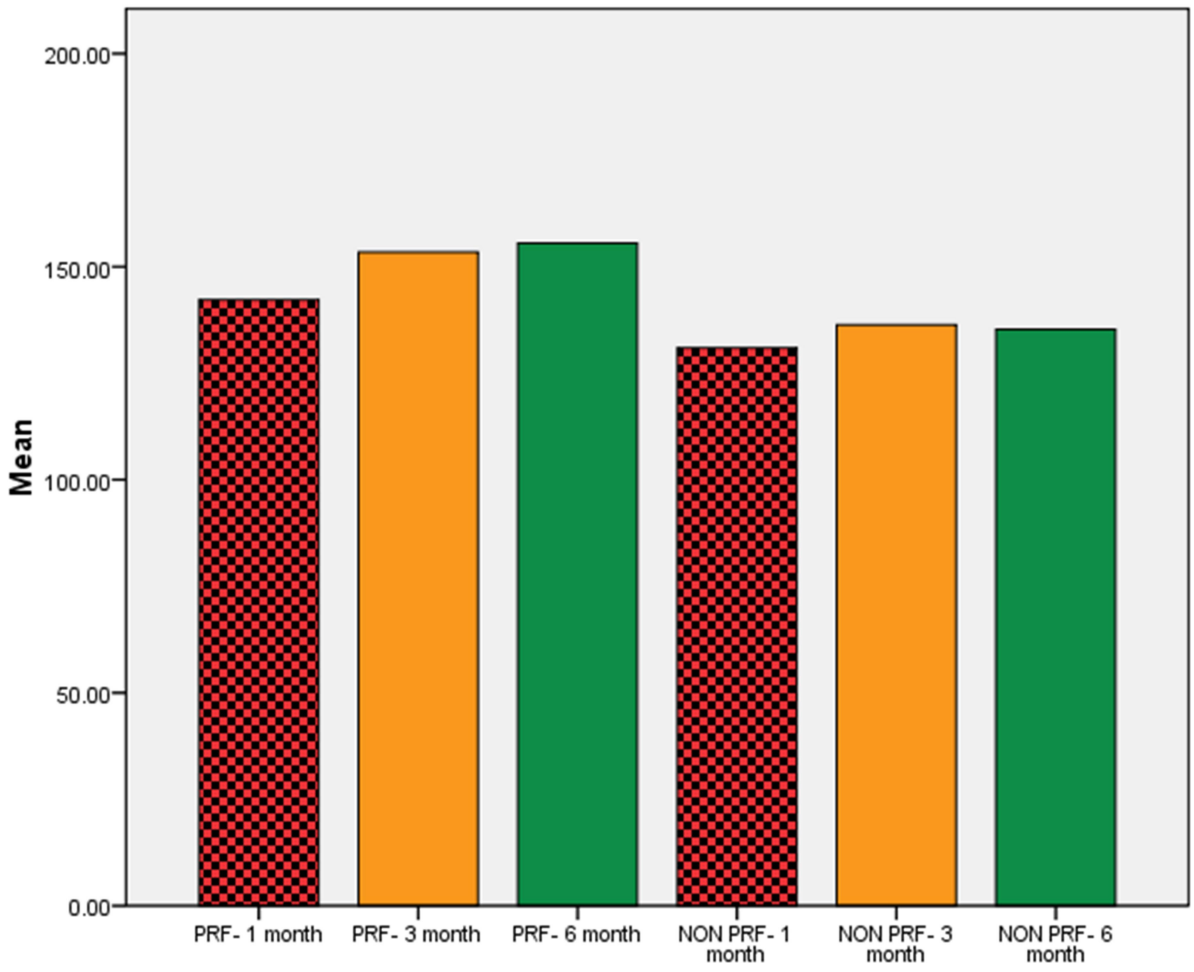
Table 7: Pair wise Comparison - T test NON-PRF

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NON PRF- 1 month	130.9305	20	21.06706	4.71074
	NON PRF- 3 month	136.3345	20	16.45848	3.68023
Pair 2	NON PRF- 1 month	130.9305	20	21.06706	4.71074
	NON PRF- 6 month	135.2955	20	12.55874	2.80822
Pair 3	NON PRF- 3 month	136.3345	20	16.45848	3.68023
	NON PRF- 6 month	135.2955	20	12.55874	2.80822

Table 8: Comparing NON-PRF using paired samples test and measure p value

Paired Samples Test									
		Paired Differences					t	df	P value (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	NON PRF- 1 month - NON PRF- 3 month	-5.40400	15.33981	3.43009	-12.58325	1.77525	-1.575	19	.132
Pair 2	NON PRF- 1 month - NON PRF- 6 month	-4.36500	15.19491	3.39769	-11.47644	2.74644	-1.285	19	.214
Pair 3	NON PRF- 3 month - NON PRF- 6 month	1.03900	12.16892	2.72105	-4.65623	6.73423	.382	19	.707

Graph


200.00
150.00
100.00
50.00
0.00
Mean
PRF- 1 month
PRF- 3 month
PRF- 6 month
NON PRF- 1 month
NON PRF- 3 month
NON PRF- 6 month

DISCUSSION

Whenever there is an injury in the body, it prepares the site for healing by humoral and cellular reactions of inflammation. Cytokines and biologically active growth and differentiation factors control the cascade of biological events. Healing is a complex biological process which results in the restoration of tissue integrity. Classically, this process of wound healing is divided into four distinct phases: hemostasis, inflammation, proliferation and tissue remodeling [(31)].

On occurrence of an injury or surgical incision, the immediate response of the body is to prevent exsanguination and promote hemostasis. Hemostasis begins with vascular constriction and fibrin clot formation. Fibrin is released from platelet and acts as an initiator for cellular response to promote wound healing. Fibrin is also responsible for early burst of fibroplasia, collagen synthesis and neovascularization. Fibrin and its degradation product, along with macrophages and monocytes circulate in the wound site and release pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) until repair is complete. Further blood loss at this stage is prevented [(1)].

Once bleeding is controlled, inflammatory cells migrate into the wound (chemotaxis) and promote the 2nd phase, the inflammatory phase. This stage of wound healing prevents infection. The inflammatory phase of wound healing will ensure that all excessive bacteria and debris from the wound is cleared. Protracted inflammation can lead to an extensive tissue damage and delay the proliferation phase. Protracted inflammation can also result in formation of a chronic wound. Lipoxins and products of arachidonic acid metabolism have anti-inflammatory properties, which dampen the immune response and allow the next phase of wound healing to arise [(1)].

Once the injuring stimulus has ceased, hemostasis has been achieved, the inflammatory response is balanced and the wound is free of debris, the proliferative stage of healing can begin to repair the wound. This complex process incorporates angiogenesis, granulation tissue formation, collagen deposition, epithelialization and wound retraction occurring simultaneously. Wounds begin to contract about seven days after injury, mediated mainly by myofibroblasts. Interactions between actin and myosin pull the cell bodies closer together decreasing the area of tissue needing to heal. Contraction occurs at a rate of 0.75 mm per day leading to shortened scars. This is influenced by numerous factors including wound shape, with linear wounds contracting fastest and circular wounds the slowest. Disorders of this phase of healing can lead to deformity and the formation of contractures [(1) (2)].

The final phase of wound healing is remodeling, which results in development of normal epithelium and scar tissue maturation. This phase involves a balance between degradation and synthesis, as the collagen and other proteins deposited in the wound become increasingly well organized. They regain a structure similar to unwounded tissue and as the scar mature the level of vascularity decreases and the scar changes from red to pink to grey with time [(1)].

Role of platelets:

Platelets play a vital role not only in hemostasis but also in wound healing. The platelets are anucleate cytoplasmic fragments derived from megakaryocytes. Platelets contain α granules and 30 bioactive proteins. After 10 minutes of platelet aggregation/clotting platelets actively secrete proteins and α granules within 1 hour. When the activation phase starts, α granules binds with the platelet plasma membrane and releases numerous amount of growth factors such as Platelet Derived Growth factor (PDGF), Transforming growth factor (TGF-β), Insulin like Growth Factor (IGF), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor

(EGF), platelet factor 4, interleukin (IL)-1, platelet-derived angiogenesis factor, platelet derived endothelial growth factor, epithelial cell growth factor and cytokines [3].

Using this concept various Platelet concentrates are made available at wound site to promote rapid and better healing and denser bone regeneration.

Role of various growth factors in wound healing process [4]:

TGF-β stimulates angiogenesis and production of fibronectin, glycosaminoglycans and collagen in connective tissue. The most important functions of TGF-β are chemotaxis and mitogenesis of osteoblast precursors.

PDGF stimulates DNA and protein synthesis, bone resorption as well as collagen and matrix formation. It acts as a potent mitogen in the serum for mesenchymal cells, including fibroblasts and smooth muscle cells. It also serves as a powerful chemoattractant for smooth muscle cells, fibroblasts, macrophages, and leukocytes.

EGF or PDEGF stimulates epidermal regeneration, promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts and enhances the effects and production of other growth factors.

PDAF stimulates vascular endothelial cells by direct or indirect actions, in which new blood vessels invade devascularized tissue. This factor is highly expressed by the induction of hypoxia.

IGF-1 stimulates cartilage growth, bone matrix formation and replication of preosteoblasts and osteoblast. IGF-1 have been isolated from macrophages in wounds and it also act as a local messenger (paracrine factor). IGF-1 in combination with PDGF enhances the rate and quality of wound healing.

PF-4 is a chemoattractant for neutrophils released from alpha granules. It may be partially responsible for the initial influx of neutrophils into the wounds. It also acts as a chemoattractant for fibroblasts and is a potent antiheparin agent.

Evolution of Platelet concentrates [3]:

In 1974, Ross et al were the first to describe the growth factors from the platelets and regenerative potential of the platelets. It was found that the growth factors were released after activation of the platelets and were trapped within the fibrin matrix. These growth factors stimulated the mitogenic response during normal wound healing.

For more than two decades, many studies were carried out to understand the physiologic properties of platelets in wound healing. These studies emphasized the therapeutic application of platelets in various forms showing varying results such as,

1) Platelets isolated from the peripheral blood can be used as autologous source of growth factors.
2) Platelet concentrates derived from the blood can be used for the prevention and treatment of bleeding due to conditions like severe thrombocytopenia, severe oral hemorrhage associated with medullary aplasia and acute leukemia.
3) Development of platelet concentrates as a bioactive surgical additive applied locally to promote wound healing.

Several components of the blood, recognized since 1990, form a part of the natural healing process and have the potential to accelerate wound healing when added to injured tissues or surgical sites. In 1990,Gibble and Ness introduced fibrin glue formed by polymerizing fibrinogen with thrombin and calcium. The fibrin glue was prepared using donor plasma. These fibrin adhesives can be derived either autologously from the patient or can be obtained commercially,

but with a risk of disease transmission in the latter one. These products can be heat-treated, thus immensely reducing, but not entirely eliminating the risk of disease transmission. Therefore, the commercially available adhesives constitute a small risk of disease transmission, such as transmission of hepatitis. Further, fibrin gel is composed of small soluble molecules, which are released too quickly to be closely built in inside the fibrin matrix during polymerization. The stability and quality of fibrin glue was low because of low concentration of fibrinogen in plasma.

Platelet Rich Plasma:

In the early 1980's, Hunt and Neighlton [(21)] described the separation of patient's blood components by centrifugation into three layers;

1. platelet-rich plasma (PRP),
2. platelet-poor plasma (PPP) and
3. Red and white blood cell layers.

PRP was prepared through double centrifugation method. Blood withdrawn from the patient is subjected to initial centrifugation at 2400rpm for 10 minutes. After separation of PPP portion and PRP from the red blood cell fraction, both were again centrifuged at 3600rpm for 15 minutes to separate the PRP from PPP [(19)].

Platelet-rich plasma gel is an autologous modification of fibrin glue. PRP obtained from autologous blood contains a concentrate of platelets. PRP deliver growth factors in high concentrations to the site of bone defect or a region requiring augmentation. These platelets have granules that contain growth factors such as TGF-β and PDGF, which promote the formation of cell and tissue involved in the wound healing and regeneration of soft and hard tissue. It also stimulates cell proliferation, angiogenesis and matrix remodeling. The growth factors are released immediately with significant reduction at 3,7,14 and 21 days and it produce transient

effect on wound healing. IGF-1 also enhances the cascade of tissue repair mechanism and it enriches the blood clot in order to fasten the wound healing and stimulate bone regeneration. As the number of platelets delivered to the site of injury increases the amount of growth factor released also increases [(7)].

The PRP coagulation process can be initiated with 10% calcium chloride and bovine thrombin. This effect causes rapid degranulation of platelets and liberation of growth factors into the surgical site. The immediate release of growth factors can only affect the immediate stages of wound healing but it will not extend to the period of time needed for bone and soft tissue regeneration [(8)].When PRP reacts with thrombin it induces fibrin clot formation, which in turn is capable of up-regulating collagen synthesis in the extracellular matrix and provides a scaffold for cellular migration and adhesion. It also stimulates the proliferation of periodontal ligament and osteoblastic cells, at the same time epithelial cell proliferation is inhibited due to its fibrinogen content [(41)].

The use of Topical Bovine Thrombin in PRP preparation may lead to risk of life threatening coagulopathies due to factor V deficiency caused by the cross-reactivity of anti-bovine factor V antibodies with human factor V following thrombin exposure (7) .

Due to the legal restrictions on blood handling new family of platelet concentrate, which is neither fibrin glue nor a classical platelet concentrate, appeared in France. This new biomaterial called platelet rich fibrin (PRF) looks like an autologous cicatricial matrix [(14)].

Platelet Rich Fibrin:

Platelet-rich fibrin (PRF) described by Choukroun et al. in France in 2001 is a second generation platelet concentrate, which allows the formation of fibrin membrane enriched with platelets, growth factors, leucocytes and cytokines. It is prepared by single stage centrifugation without adding any additives or anticoagulant [(26)].

Coagulation starts immediately during centrifugation and three parts appear in the tube:

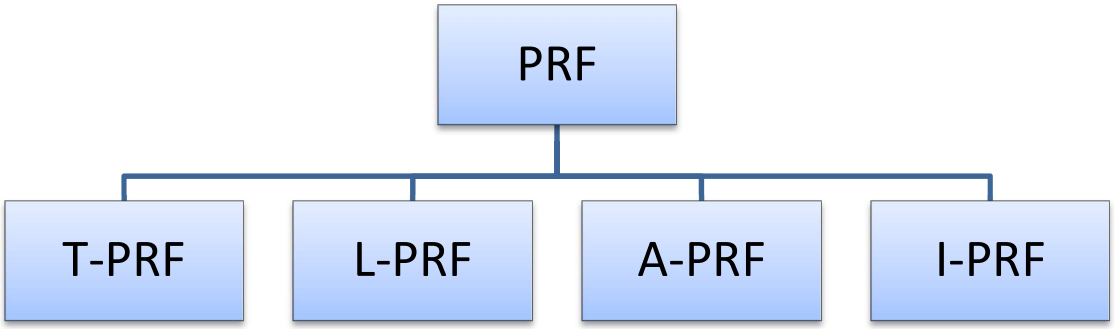
- Acellular plasma at the top and
- PRF clot in the middle and
- RBC at the bottom.

While separating the PRF clot, a small RBC layer at the junction of PRF clot has to be retained because more concentration of platelet and leucocyte are located intermediately between RBC and fibrin clot represented by buffy coat on PRF surface. This PRF clot can be transformed into a membrane by squeezing the clot using sterile gauze [(26)].

Classification of PRF:

PRF can be classified into four types, depending on the leucocyte and fibrin content, namely [(50)]:

- Titanium prepared, platelet rich fibrin (T-PRF),
- Leucocyte and platelet-rich fibrin (L-PRF),
- Advance platelet-rich fibrin (A-PRF),
- Injectable platelet-rich fibrin (I-PRF).



Titanium-prepared, platelet rich fibrin (T-PRF):

T-PRF collection protocol is similar to the standard PRF protocol and the difference lies in the usage of titanium tubes instead of glass-evacuated collection tubes. Problem with the glass-evacuated collection tube is the possibility of health hazard from silica activators. Small fraction of the glass tube's silica particles remain suspended in the fibrin and platelet-poor layers of plasma, which might reach the patient when PRF is used for treatment. T-PRF is more woven and thick polymerized fibrin due to the biocompatibility and hemocompatibility of titanium.

The centrifugation protocol of T-PRF is 2800rpm for 12mins [(50), (49)].

Leucocyte and platelet-rich fibrin (L-PRF)

The leukocyte and platelet rich fibrin (L-PRF) is a second generation platelet concentrate. The fibrin architecture of L-PRF serves as a reservoir for growth factors such as PDGF and TGF-β and also cytokines. L-PRF has a favourable effect on proliferation and differentiation of osteoblast cells through the release of its growth factors. L-PRF also stimulates osteoblast adhesion and regulates the collagen protein production [(51)].

L-PRF acts as an excellent scaffold, facilitating a slow release over 7 days. The L-PRF also has a role in the regulation of inflammation and prevention of infection due to high content

of leukocytes present in the thin layer near the red blood cells. L-PRF also stimulates fibroblast, migration and angiogenesis because its fibrin structure can be penetrated by fibroblasts.
The centrifugation protocol of L-PRF is 2700rpm for 12mins [51), (50].

Advance platelet-rich fibrin (A-PRF)

A-PRF follows a similar collection protocol to that of standard PRF protocol and the difference lies in blood centrifuge specification. The centrifugation protocol of A-PRF is 1500 rpm for 14mins, compared to that of standard PRF which is 2700rpm for 12mins.With decrease in centrifugation rpm and increase in time, enhanced neutrophil granulocytes in the distal part of the clot are obtained. Neutrophil granulocytes contribute to monocyte differentiation into macrophages, which in turn releases higher concentration of growth factors.

PRF clots formed with A-PRF centrifugation protocol has a loose structure with more inter fibrous space and more cells in distal part of fibrin clot. The distribution of platelets in A-PRF is far away from the buffy coat (BC) compared to L-PRF [(50)].

Injectable platelet-rich fibrin (I-PRF)

I-PRF is obtainable in a short duration and it is a new alternative to the platelet aggregate. Blood samples are collected in uncoated tubes without any additives. Duration of centrifuge is two minutes at 3300 rpm. Upon termination of this process, an orange color area (i-PRF) is observed in the tube. I-PRF is a viable option in regenerative procedures as it is autogenous and decreases the chances of adverse reactions to the implanted material. Also the effective bonding of i-PRF with biomaterials makes it an alternative to PRP in cases of bone grafting and bone regeneration. The platelet aggregates from i-PRF is finding its importance in different areas of Medicine and Dentistry, especially in orthopaedics and in plastic surgery [(50)].

Many growth factors are released from PRF, such as platelet-derived growth factor (PDGF), Transforming growth factor (TGF-b), and vascular endothelial growth factor (VEGF). PDGF and TGF-β are released from platelets upon activation with thrombin. In combination with IGF, which is located in plasma, PRF supports bone regeneration. These growth factors also promote cell proliferation, cell differentiation, motility and matrix synthesis either alone or upon binding with specific cell surface receptor [(19)].

In addition to that, PRF slows down the blood activation process, which could induce an increased leukocyte degranulation and cytokine release from proinflammatory mediators, such as interleukin (IL)-1b, IL-6 and tumor necrosis factor-α, to anti-inflammatory cytokines, such as IL-4 [(41)]. PRF is made up of a fibrin matrix polymerized in a tetramolecular structure with the incorporation of platelets, leukocyte, and cytokines; and circulating stem cells. PRF matrix enmeshes glycosaminoglycans (heparin, hyaluronic acid) from blood and platelets. Histologically fibrin follows the fibrillary architecture and has a greater capacity to support cell migrations and healing processes [(10)].

The platelet cytokines play three main roles in initial healing mechanism

1. It stimulates cell migration and proliferation by PDGFs.
2. Induce fibrin matrix remodelling.
3. Secretion of a cicatricial collagen matrix by TGF-β.

The cytokines are small soluble molecules and it is found neither in the supernatant nor in the exudate. Even after serum exudation the cytokines are trapped in the PRF fibrin matrix, which could incorporate these molecules in the fibrin polymer architecture [(15)].

The PRF formation is a natural and progressive polymerization initiated during centrifugation. The fibrin network is formed by homogeneous 3-dimensional organization. This

is confirmed by alcian blue staining. This polymerization signifies increased incorporation of the circulating cytokines into the fibrin meshes (intrinsic cytokines) and it increases lifespan of cytokines, because it will be released and used only at the time of initial cicatricial matrix remodeling (long-term effect). The cytokines are maintained in a convenient media till it is released [(15)].

The released platelet cytokines are trapped in the colloidal suspension between the fibrin network meshes during gelling. Their physiologic elimination will be fast and it shares the cytokines fibrin synergies from PRF on healing process. PRF also organizes as a dense fibrin scaffold with a high number of leukocytes concentrated in one part of the clot, with a specific slow release of growth factors and glycoproteins for at least one week and up to 28 days. This means that the membrane stimulates its environment for a significant period of time during wound healing [(35)].

PRF has a natural fibrin framework and can protect growth factors from proteolysis. Thus, growth factors can keep their activity for a longer period relatively and stimulate tissue regeneration effectively. It also stimulates human gingival fibroblast, osteoblast and PDLF cell proliferation as mitogen [(9)].In PRF, the conversion of fibrinogen into fibrin takes place slowly with small quantities of physiologically available low concentration of thrombin present in the blood sample. This leads to slow polymerization process, very favorable to the healing process. The slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains within the fibrin meshes [(52)].

PRF has many advantages over PRP. It eliminates the redundant process of adding anticoagulant as well as the need to neutralize it. It avoids the addition of bovine-derived thrombin to promote conversion of fibrinogen to fibrin. The elimination of these steps in PRF is

to reduce the biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin [41].

Soft tissue changes: PRF accelerates the initial phase of healing and epithelialization of soft tissue by the release of growth factors such as PDGF, TGF-β, and VEGF in the socket and it stimulates angiogenesis and granulation tissue formation. PRF not only improves the healing alone but also improves keratinization of the gingiva [31].

Cartilage repair: When the PRF is mixed with cartilage granules it provides cell differentiation and cell growth. PRF release fibrin to support the cytokine enmeshment, cellular migration and stem cell trapping. The cytokines contain interleukin to promote the normal immune function. When the scaffold is seeded with chondrocytes it improves the healing of cartilage defect and this scaffold improves the re-differentiation capacity of chondrocytes by providing a mechanical stability [33].

Bone grafts: When PRF mixed with osseomold graft is placed into the intra bony defect, it released platelets, leucocytes, circulating stem cells, growth factors and cytokines during fibrin matrix remodelling. The PRF organized as a dense fibrin scaffold with high number of leucocyte concentrated with slow release of growth factors such as TGF-β, VEGF, PDGF and glycoproteins [10].

Sinus lift and implant placement: PRF is used as a sole filling material in the sinus lift procedure to improve the healing of schneiderian membrane and to stabilize the bone volume around the implant. The slow release of growth factor from PRF can easily replace the use of xenogenic graft and collagen membrane [23].

Moraschini et al [6] revealed PRF has no significant difference in hard tissue regeneration when compared to PRF and PRP. In their histological study they showed that contaminated sockets will heal slowly when compared to the sockets free of contamination.

Volker Gassling et al [39] compared bone regeneration following PRF placement with collagen membrane placement following maxillary sinus augmentation. In both the sides good bone formation occurred and the mean value on PRF side was 17% when compared to collagen membrane which is 17.2%.

Bahadir Gurbuzer et al [28] revealed that scintigraphically detectable bone formation between the PRF and Non-PRF sockets were statistically nonsignificant.

We selected 25 patients (Annexure 1) both male and female in the age group of 18-35 years who came for prophylactic surgical removal of bilateral mandibular impacted third molar to the department of Oral and Maxillofacial Surgery in Sri Ramakrishna Dental College and Hospital for our study. The study was approved by our institutional Ethical committee. Of the 25 patients only 20 patients who came for regular follow up (Annexure 2) was taken for study. In our study we evaluated the alveolar bone regeneration in the socket placed with PRF and without PRF after surgical removal of bilateral impacted mandibular third molar tooth.

Inclusion criteria for our study were that the patients should be free of any symptoms pertaining to the surgical field. The patients' medical status should come under ASA I and II category. Patients with diabetes mellitus and any other medical condition that affect wound healing were excluded from our study. Our exclusion criteria also included local conditions like dental caries, pulpitis, pericoronitis or any periapical lesions.

Each of the patient was categorized into two groups: PRF group (test side) and non- PRF group (control side). The side chosen as test side was random. Surgery was performed to remove both the mandibular third molar tooth in the same visit by the same surgeon.

In the test side after the surgical removal of third molar, the PRF was placed in the socket followed by routine primary closure. In the control side after surgical removal of third molar, the primary closure was done. PRF side or test side surgery was performed first in order to decrease the invitro timing of the freshly prepared PRF.

Similar studies were done by Olufemi et al [32], Joy R.Das et al [7], Guilherme de Marco et al [42] ,Ruchi Pathak et al [5] , Tejesh Yelamali et al [52] and Bahadir et al [28], to evaluate the efficiency of PRF in bone healing by comparing a test group where the PRF was placed in extracted socket with a control group allowed to heal routinely. Pushkar et al [22], Ronaldo et al [36] and Gilberto et al [53] used sockets of bilateral surgical removal of mandibular third molars. Patient was recalled for follow up after 1st month, 3rd month and 6th month postoperatively. Orthopantomograms (OPG) were taken during each of the three visits.

Pushkar et al [22], Joy R.Das et al [7], Gilberto et al [53] and Tejesh Yelamali et al [52] also used OPG to measure the difference in density between test and control side. OPG was input into the Image Processing Toolbox™ of Matlab software. Pushkar et al [22], Joy R.Das et al [7] used Adobe Photoshop 7.0 software to measure the density of bone in the extracted socket. Ronaldo et al [36] used HLImage 97++ software to measure the density of bone in the extracted socket. Tejesh Yelamali et al [52] used Adobe photoshop CS software to measure the density of bone in the extracted socket. Abbas Haghighat et al [54] used Photoshop 8 software to measure the density of bone in the extracted socket.

Image Processing Toolbox™ of Matlab provides a comprehensive set of standard algorithms and workflow apps for image processing, analysis tasks such as statistical analysis and property measurement, visualization and algorithm development. We can also perform image segmentation, image enhancement, noise reduction, geometric transformations, image registration and 3D image processing using this toolbox. We used this tool box to extract density information from test side and control side sockets.

Algorithm developed in Matlab to measure the density from an OPG image is as follows:

1. OPG image taken from the patient to be read into the image processing tool box.
2. Image to be converted from Red-Green-Blue (RGB) format to gray scale (Black/White) format.
3. Pixel values to be extracted from PRF region and Non-PRF region separately.
4. The average of the extracted pixel values from test side and control side is computed.
5. Each pixel value obtained conveys the intensity of that region. Intensity value ranges from 0 to 255, 255 indicating the densest region. Hence average of the pixel value conveys the density over that region.
6. Later, the density values of test side and control side in 1st, 3rd and 6th month were compared in order to see the improvement with PRF technique.

In our study, 2 patients had a postoperative complication of paraesthesia on the control side and 1 patient had paraesthesia on test side which reduced after two weeks of follow up. No other patient had any other postoperative complications immediately or within 6 months of follow up. We used paired t test and Repeated ANOVA as statistical tool to compare the values. Joy R.Das et al [(7),] Guilherme de Marco et al [(42)] and Tejesh Yelamali et al [(52)] used similar statistical analysis to find the significance of their study.

Statistical density comparison between test side and control side are:

The 1st month follow-up showed significant difference in regeneration in PRF side compared to non-PRF side for 15 patients with a p value p=0.06. During 3rd month follow-up significant regeneration was observed in PRF side over non-PRF side for 19 patients with a p value p=0.00.

At the 6th month follow-up significant difference between PRF and non-PRF side is seen in all the 20 patients with a p value of p=0.00. Using the statistical analysis of paired test a significant difference was noted on 1st, 3rd and 6thmonth post operatively on PRF side compared to non-PRF side.

In the Repeated measure ANOVA test, a significant difference was noted on 1st, 3rd and 6th month of PRF side post operatively.

Lokman Onur Uyanik et al [48] and Tejesh Yelamali et al [52] showed good results similar to our study. These studies emphasize that there were significant improvement in bone healing following PRF placement. Bahadir Gurbuzer [28] studies emphasize that there was no significant difference in bone healing between PRF and non PRF side.

SUMMARY

A clinical study was done to evaluate the efficacy of Platelet rich fibrin (PRF) as bone regenerative material in the extracted socket following surgical removal of bilateral impacted mandibular third molars on the same session at Sri Ramakrishna Dental College & Hospital, Coimbatore.

PRF was prepared from blood samples taken from patient's own venous blood and centrifuged at 3000rpm for 10 minutes. The prepared PRF was placed in one of the extracted sockets, chosen randomly without any criteria and the contralateral socket was taken as control.

The bone density of PRF and non-PRF side was evaluated on OPG using Matlab software at 1st month, 3rd month and 6th month postoperatively.

Our goals was to enhance the surgical wound healing following the surgical extraction of mandibular third molar and improve the bone regeneration during the post-operative period.

The radiographic measurements were recorded at 1st, 3rd and 6th months post operatively, which showed the following results:

- There was a significant difference noted on 1st month post operatively ($p=0.061>5\%$) on PRF side compared to non-PRF side.
- A highly significant difference was noted on 3rd month post operatively ($p=0.000<1\%$) on PRF side compared to non-PRF side.
- And also a highly significant difference was noted on 6th month post operatively ($p=0.000<1\%$) on PRF side compared to non-PRF side.

The mean difference between the two sides was statistically significant ($p<0.05$) during the 6 months follow up.

This increase in the bone density signifies and highlights the use of PRF, and can be certainly considered as a valid method in accelerating soft and hard tissue regeneration within 6 months. Although the comparison of two groups reveals statistically significant difference, the hypothesis of concept of PRF and the bone regeneration capacity cannot be rejected.

CONCLUSION

The conclusion drawn from our study is as follows:

- PRF is a natural and optimized blood clot that appears to be adequate in improving the alveolar bone regeneration in the extracted sockets.
- The procedure for the preparation of PRF is simple, safe and inexpensive for the patient.
- PRF improves and fastens the bone healing in the extracted socket of impacted mandibular third molar within 6 months after surgery when compared to non-PRF side.

PRF still continues to be a biological tool which has a potential that calls for an extensive research in various types of surgeries. The clinical applications of PRF uses are numerous and should be further explored in the coming years in the surgical field.

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ANNEXURES

TABLE-1

S.NO	DATE	OP.NO	NAME	AGE/SEX	TYPE OF IMPACTION		TEST SIDE	CONTROL SIDE	FOLLOW UP
					RIGHT	LEFT			
1.	18.12.15	15742	SWETHA SHARAN	21/F	HORIZONTAL	HORIZONTAL	38	48	YES
2.	07.01.16	28805	ABIRAMI	25/F	DISTOANGULAR	MESIOANGULAR	38	48	YES
3.	25.01.16	01403	NANDHINE	20/F	HORIZONTAL	MESIOANGULAR	38	48	YES
4.	27.01.16	01605	SABARISH	27/M	HORIZONTAL	HORIZONTAL	48	38	
5.	29.01.16	01728	JOSHVA	21/M	MESIOANGULAR	HORIZONTAL	48	38	YES
6.	05.02.16	02214	DHIVYA BHARATHI	21/F	MESIOANGULAR	MESIOANGULAR	48	38	YES
7.	12.03.16	05304	VIGNESH PRABHU	25/M	MESIOANGULAR	MESIOANGULAR	48	38	NO
8.	15.03.16	03782	AZHAZAN	21/M	HORIZONTAL	HORIZONTAL	48	38	YES
9.	08.04.16	05496	VIJAYA LAKSHMI	25/F	VERTICAL	HORIZONTAL	48	38	YES
10.	26.08.16	19166	SUKUMAR	23/M	MESIOANGULAR	VERTICAL	48	38	NO
11.	22.09.16	14590	BALAJI	18/M	MESIOANGULAR	MESIOANGULAR	48	38	YES
12.	18.11.16	01148	UDHYA	25/F	HORIZONTAL	HORIZONTAL	48	38	YES
13.	05.12.16	18220	MANOJ	25/M	HORIZONTAL	HORIZONTAL	38	48	NO
14.	31.12.16	05294	PRAVEEN	25/M	DISTOANGULAR	DISTOANGULAR	38	48	YES
15.	02.01.17	30271	ANGEL SHARLIN	20/F	MESIOANGULAR	MESIOANGULAR	38	48	YES
16.	13.01.17	18445	LOGESH	25/M	HORIZONTAL	HORIZONTAL	48	38	YES
17.	03.02.17	01763	PRAVEEN KUMAR	25/M	HORIZONTAL	HORIZONTAL	38	48	YES
18.	06.02.17	02093	PRABAKARAN	23/M	HORIZONTAL	HORIZONTAL	38	48	YES
19.	09.02.17	21598	RAMESWAR	24/M	HORIZONTAL	HORIZONTAL	38	48	YES
20.	27.02.17	03856	KARPAGAM	22/F	HORIZONTAL	HORIZONTAL	38	48	YES
21.	15.03.17	05457	MALATHI	32/F	HORIZONTAL	HORIZONTAL	38	48	YES
22.	11.04.17	05133	PRATHEESH	22/M	HORIZONTAL	HORIZONTAL	38	48	YES
23.	18.04.17	07548	MANIKANDAN	24/M	HORIZONTAL	DISTOANGULAR	48	38	NO
24.	21.04.17	07833	ARUN KUMAR	27/M	HORIZONTAL	HORIZONTAL	38	48	NO
25.	26.04.17	08188	PUNITHA	35/F	HORIZONTAL	HORIZONTAL	38	48	YES

TABLE- 2

S.NO	NAME	TEST SIDE			CONTROL SIDE		
		1MONTH	3MONTH	6MONTH	1MONTH	3MONTH	6MONTH
1	SWETHA	144.66	153.75	144	127.27	137	137.88
2	ABIRAMI	117.35	165.07	177.81	159.45	169.92	136.6
3	NANDHINI	154.09	152.35	130.94	110.43	107.67	113.83
4	JOSHUA	140.7	155.98	145.3	128.49	149.4	122.3
5	DHIVYA	145.51	138.72	138.95	134.98	136.35	137.83
6	AZAHGAN	147.74	162.3	172.97	151.27	147.71	137.88
7	VIJAYALAK	112.93	131.73	140.26	103.06	119.3	131.1
8	SUKUMAR	149.9	176.64	173.74	149.47	127.05	148.21
9	BALAJI	169.69	157	172.53	137.19	134.96	140.97
10	UDHYA	161.71	158.37	172.79	107.57	109.82	110.66
11	PRAVEEN	108.71	146.54	142.09	100.23	139.15	141.15
12	ANGEL	152.36	156.73	157.71	139.94	134.54	137.84
13	LOGESH	122.38	146.18	155.75	135.65	133.12	130.95
14	PRAVEEN	146.46	149.12	150.15	127	129.13	130.19
15	PRABAKA	156.64	154.99	158.16	135.89	162.17	152.45
16	RAMESWA	141.05	133.3	136.15	104.24	127.75	126.67
17	KARPAGA	132.02	166.42	168.34	181.38	165.08	164.34
18	MALATHI	161.62	154.81	162.21	149.41	135.56	145.67
19	PRATHEES	148.53	149.23	151.45	114.96	123.23	124.51
20	PUNITHA	130.29	159.12	158.97	120.73	137.78	134.88

One-Sample Kolmogorov-Smirnov Test

		PRF- 1 month	PRF- 3 month	PRF- 6 month	NON PRF- 1 month	NON PRF- 3 month	NON PRF- 6 month
N		20	20	20	20	20	20
Normal Parameters[a,,b]	Mean	142.2170	153.4175	155.5135	130.9305	136.3345	135.2955
	Std. Deviation	16.81088	10.86490	14.33338	21.06706	16.45848	12.55874
Most Extreme Differences	Absolute	.164	.112	.132	.085	.182	.121
	Positive	.081	.100	.112	.085	.182	.121
	Negative	-.164	-.112	-.132	-.076	-.092	-.092
Kolmogorov-Smirnov Z		.734	.502	.592	.379	.814	.539
P value		.655	.963	.874	.999	.521	.933

a. Test distribution is Normal.

b. Calculated from data.

SRI RAMAKRISHNA DENTALCOLLEGE & HOSPITAL

SNR COLLEGE ROAD, COIMBATORE- 641006.

CASE RECORD

PATIENT OP NO: STUDY NO:

NAME: AGE/SEX:

ADDRESS: OCCUPATION:

CHIEF COMPLAINT: VITAL SIGN:

PULSE	
BP	
TEMPERATURE	
RESPIRATORY RATE	

HISTORY OF PRESENTING ILLNESS:

PAST MEDICAL HISTORY:

PAST DENTAL HISTORY:

PERSONAL HISTORY:

GENERAL EXAMINATION

GAIT: BUILT:

LOCAL EXAMINATION:

EXTRA ORAL EXAMINATION

FACIAL SYMMETRY:

TMJ EXAMINATION:

LYMPH NODES:

MOUTH OPENING:

INTRA ORAL EXAMINATION:

SOFT TISSUES:

HARD TISSUE:

STATUS OF THIRD MOLAR:

INVESTIGATION:

IOPA

PANTOMOGRAM

DIAGNOSIS:

TREATMENT PLAN:

III MOLAR ASSESSMENT

PELL & GREGORY'S CLASSIFICATION:

	RIGHT	LEFT
CLASS		
POSITION		
ANGULATION		

WAR LINES:

RIGHT: LEFT:

WHARFE's ASSESMENT :

	RIGHT	LEFT
WINTER'sCLASSIFICATION		
HEIGHT		
ANGULATION		
ROOT SHAPE		
FOLLICLE		
EXIT PATH		
TOTAL SCORE		

PEDERSON'S DIFFICULTY INDEX:

RIGHT: LEFT:

TREATMENT DONE: RIGHT LEFT

PROCEDURE :

COMPLICATIONS IF ANY:

DURATION :

MEDICATIONS:

Signature of the operator

INFORMED CONSENT FORM

I _____(name of the patient) hereby authorize the doctor in charge to administer treatment and to perform any such procedure as may be deemed necessary or advisible in diagnosis and treatment. I have been explained about my oral conditions and the treatment planned.

I hereby give full consent to undergo surgical removal of my impacted tooth under local anesthesia and placement of Autologous Platelet Rich Fibrin from my own blood in one of the extracted socket. The doctor has explained to me about the advantages, disadvantages and the risk associated with the procedure in an understandable language.

Date:

Place: Signature of the patient Signature of the doctor

POST OPERATIVE REVIEW:

	RIGHT	LEFT	SIGN
2nd DAY			
1 WEEK			
1 MONTH			
3 MONTH			
6 MONTH			